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VOLUME 32 APRIL 1954 NUMBER 2

# SOMATIC CHROMOSOMES OF HIGHER DIPTERA

## III. INTERSPECIFIC AND INTRASPECIFIC VARIATION IN THE GENUS *HYLEMYA*[1]

BY J. W. BOYES[2]

## Abstract

Analyses of the somatic complements of *Hylemya antiqua*, *H. brassicae*, *H. cilicrura*, *H. crucifera*, *H. floralis*, *H. fugax*, *H. planipalpus*, and *H. trichodactyla* (?) have been made following techniques described in the first paper of this series. All species, except *H. fugax*, have 12 chromosomes. These 12-chromosome species fall naturally into three main groups differing in average arm ratios and in other respects. *H. fugax* males have 13 chromosomes and females have 14, both including five pairs of submetacentric autosomes. One to three supernumerary chromosomes were found in a few larvae of *H. cilicrura*. Small intraspecific differences were found between collections of *H. brassicae* from different geographic locations. The results with this genus emphasize the inaccuracy of referring to complements which look similar as the same or identical.

## Introduction

The *Hylemya* species of the family Anthomyiidae are Diptera of considerable economic importance in many parts of the world. In Canada the larvae of a number of species attack turnip, cabbage, radish, etc. so successfully that they are presently the subject of many investigations. Obviously the identification of larval stages is very important in the development of control measures and Brooks (4) has recently published a valuable key, by means of which many larval specimens can be identified. Difficulties in identification of certain larval specimens remain and accordingly it was suggested by Dr. A. Wilkes and Mr. George Wishart, of the Laboratory of Entomology, at Belleville, Ontario, that careful analyses of the somatic chromosomes of root maggots in this genus be undertaken.

## Materials and Methods

Larval specimens were collected from many locations in Canada and the British Isles through the courtesy of many officers of the Division of Entomology, Science Service, Ottawa. Mr. George Wishart confirmed the

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*Contribution from the Department of Genetics, McGill University, Montreal, Que., with the co-operation of the Laboratory of Entomology, Belleville, Ont.*
[2] *Associate Professor and Chairman, Department of Genetics, McGill University, Montreal, Que.*

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identification of nearly all larvae used and some of them were rechecked by Mr. A. R. Brooks, of the Systematic Unit, Division of Entomology, Science Service, Saskatoon. The identification of adults reared from nearly all of these collections was confirmed by Mr. J. F. McAlpine, also of the Systematic Unit.

Data regarding the sources of the *Hylemya* collections studied and the host plants on which the larvae were collected have been recorded in Table I. The St. Jean collection of *H. antiqua* was from laboratory reared stock supplied by Mr. J. P. Perron (Division of Entomology, Science Service, St. Jean, P.Q.); other collections were from plants in the field.

TABLE I

SPECIES, HOSTS, AND SOURCES OF LARVAE STUDIED

| Species | Source of collection | Host plant |
|---|---|---|
| *Hylemya antiqua* Mg. | (1) St. Jean, Quebec | Onion |
| | (2) Belleville, Ontario | Onion |
| *Hylemya brassicae* (Bouché) | (1) Belleville, Ontario | Radish |
| | (2) Belleville, Ontario | Cabbage |
| | (3) *Cloverdale, B.C. | Radish |
| | (4) Charlottetown, P.E.I. | Turnip |
| | (5) Guelph, Ontario | Turnip |
| | (6) Prestwick, Scotland | Brussels sprouts |
| | (7) St. Johns, Newfoundland | Turnip |
| *Hylemya cana* Macquart | (1) Belleville, Ontario | Unknown |
| *Hylemya cilicrura* (Rond.) | (1) Brandon, Manitoba | Turnip |
| | (2) **Belleville, Ontario | Cabbage |
| | (3) **Cloverdale, B.C. | Radish |
| *Hylemya crucifera* Huck. | (1) Brandon, Manitoba | Turnip |
| | (2) Saskatoon, Saskatchewan | Turnip |
| *Hylemya floralis* (Fall.) | (1) Elgin, Scotland | Unknown |
| *Hylemya fugax* (Mg.) | (1) Wye, England | Rotting cabbage |
| *Hylemya planipalpus* (Stein) | (1) Brandon, Manitoba | Radish |
| *Hylemya trichodactyla* (Rond.) | (1) **Charlottetown, P.E.I. | Potato |
| | (2) **Guelph, Ontario | Turnip |
| | (3) **Cloverdale, B.C. | Radish |

** Twelve adults from this collection were identified as; nine H. brassicae, two H. planipalpus, and one Hylemya sp. but Brooks considered the larvae studied to be H. planipalpus.*

*** Identification tentative, see text.*

Larval brains were dissected out just before pupation at which time mitoses were usually found in satisfactory numbers. The methods of dissection, preparation of slides for study, and analysis of the chromosome complements were, in general, as described in the first two papers of this series (2, 3). The following modifications should, however, be noted:

(1) After staining the brains *in toto* in iron-aceto-carmine for 25 min., they were transferred directly to the Venetian-turpentine mixture in which they were squashed and which served as the mounting medium.

(2) The *X*- and *Y*-chromosomes, which are small and usually of nearly the same size, were regularly both included as Pair I. Thus the values for total complement length (TCL) represent the true total length of all chromosomes in the complement with no adjustment for males. This does not apply to *Hylemya fugax* in which adjustments for male complements were made according to the procedure previously used.

(3) In analysis of complements of all species except *Hylemya fugax* (see below), the small secondary constrictions in the *X*- and *Y*-chromosomes, even when not observed, were regularly assigned a value of 0.05 cm. when measured on the drawings. This is consistent with previous procedure but may seem unsatisfactory for such small acrocentric chromosomes. If one considers that no correction is applied for the kinetochore which appears to be terminal (i.e. no short arm has been seen), this value seems of justifiable magnitude.

(4) In analyzing complements of *H. fugax* the submedial secondary constriction in $X_1$ (Pair $I_1$) and the kinetochore of the *Y*-chromosome were regularly assigned a value of 0.10 cm. when the chromosomes were measured in the drawings. The medial secondary constriction in the small acrocentric $X_2$-chromosome (Pair $I_2$) was assigned a value of 0.05 cm.

The complements of the species studied have been represented in Figs. 3–9. In these figures the small pair of presumed sex chromosomes (Pair I) is followed by the autosomal pairs numbered in order of increasing size. Kinetochores are shown as V-shaped indentations on both sides of the chromosomes; thus when the kinetochore is at, or very near, the end, the chromosome is pointed. Conspicuous and frequently observed secondary constrictions are indicated by narrow white bands across the chromosome at the proper position. Other secondary constrictions, only occasionally found, are indicated by an arrow on one side of the chromosome at approximately the correct position.

## Descriptions of Somatic Metaphase Complements

Twelve chromosomes were regularly present in larvae of all species studied, with the exception of *H. fugax* and a few individuals of *H. cilicrura* which had supernumeraries. Thus normally a complement consisted of a small acrocentric pair presumed to be the sex chromosomes and five pairs of autosomes. *H. fugax* males have 13 and females have 14 chromosomes, both including five pairs of submetacentric autosomes. In addition to these numerical differences there are extensive morphological differences in the somatic complements on the basis of which the 12-chromosome group has been subdivided.

The species with 12 chromosomes regularly have a small pair of chromosomes, considered to be the sex chromosomes for the following reasons: (*a*) although the two are very similar in size in all individuals of a species, in some

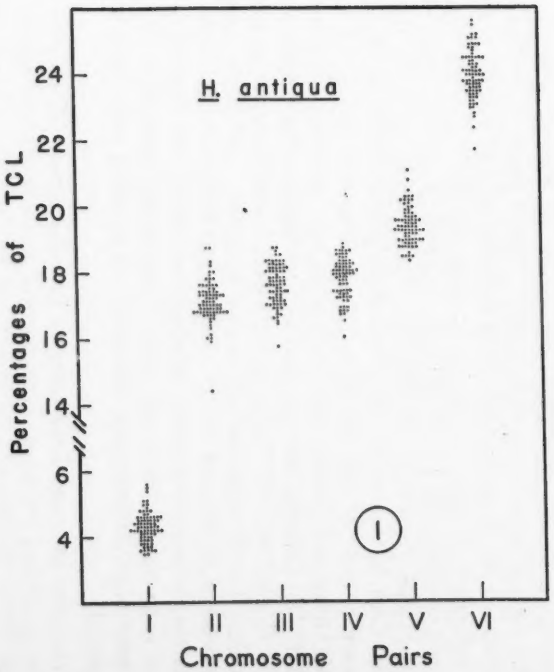
individuals they differ in morphology (see details later); (*b*) two small, positively heteropycnotic masses can be seen in some metabolic nuclei; (*c*) from their observed association with the nucleolus in late prophase they obviously contain nucleolus organizers; and (*d*) there is nothing to indicate that any other pair should be regarded as the sex chromosomes. Larvae possessing a heteromorphic pair of the small chromosomes have been assumed to be males, an assumption supported by observed slight differences between the two small chromosomes during meiosis in male pupae of *H. antiqua*. The submedial secondary constrictions differ slightly in location in the chromosomes considered to be *X* and *Y* but they are not detectable in many cases. Therefore, it was decided to take the combined length of the two smallest chromosomes as the length of Pair I (previously determined by using twice the single *X* in males or both *X*'s in females). This has introduced a very slight error proportional to the length differences between the *X*- and *Y*-chromosomes.

*Hylemya antiqua* Mg.

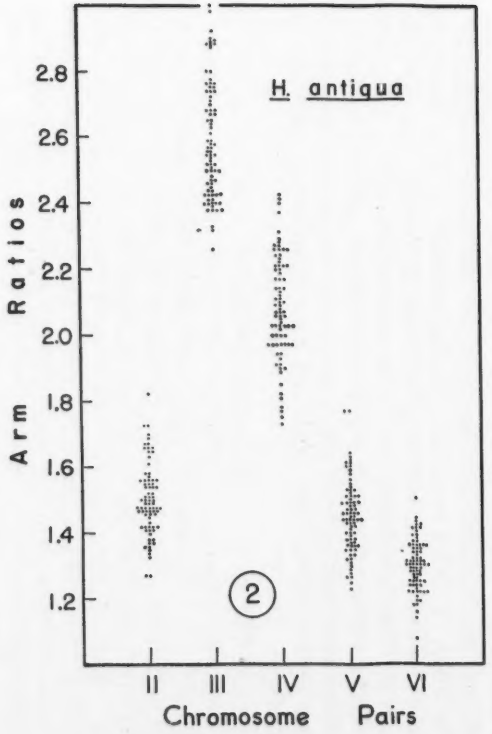
The complements of 81 cells from 33 larvae of this species have been analyzed. Sixty came from laboratory stocks reared on onion, at St. Jean, Quebec. The other 21 came from field collections of larvae on onion at Belleville, Ontario. Without exception all larvae had 12 chromosomes in each complement analyzed (Text-fig. 3 and Plate I, Fig. 1). Results are shown graphically in Text-figs. 1 and 2. The results of separate analyses for the St. Jean and Belleville collections were in such close agreement that it was decided to combine them for presentation in Text-figs. 1 and 2 and Table VII.

As previously indicated, the acrocentric presumed sex Pair I is small and quite distinct in size. The *X* and *Y* are very similar in size and thus difficult to distinguish. One sex chromosome (considered to be the *Y*) has, in some individuals, both a submedial and a subterminal secondary constriction and is slightly longer than its homologue. Pairs II and III differ slightly in length (see Table VII) but their arm ratios differ strikingly and accordingly they are distinguishable with certainty. Pairs III and IV differ even less in length whereas their arm ratios are quite distinct. Pairs IV and V differ slightly more in length but are decidedly different in their arm ratios. On the other hand Pairs V and VI differ so much that their percentages of the TCL do not overlap whereas their arm ratios differ only slightly. The above differences make assignment to pair reasonably accurate.

Secondary constrictions supply confirmatory evidence in assigning certain chromosomes to their proper pair. The short arm of Pair II has a submedial secondary constriction which, though somewhat variable in its position and only observed in about 20% of the complements, divides the arm into proximal and distal segments with the former averaging 1.40 times the length of the latter. The short arm of Pair III is similarly divided with the proximal




TEXT-FIG. 1. *H. antiqua*, percentages of TCL, results of analyses of 80 complements.




TEXT-FIG. 2. *H. antiqua*, arm ratios, results of analyses of 80 complements.

segment averaging 0.90 times the distal segment. This ratio was determinable in about a third of the complements analyzed. Also the short arm of Pair IV is similarly divided, as seen in about one quarter of the complements analyzed, into proximal and distal segments with the former averaging 1.15 times the latter. Pair VI has a secondary constriction, usually detectable, in the long arm giving a proximal segment averaging 3.9 times the distal and another in the short arm giving a proximal segment averaging 3.3 times the distal. Other achromatic bands which may indicate additional secondary constrictions have been seen in various positions in several chromosomes but, since they are irregular in appearance and inconsistent in location, they are of little value.

The average TCL for 80 complements (60 from St. Jean and the first 20 of the 21 from Belleville) was 85.35 $\mu$ with 64.3 $\mu$ as the lowest and 130.2 $\mu$ as the highest. It is apparent that pairs with higher arm ratios have higher standard deviations. Pair I is more variable in length than the autosomal pairs, perhaps because this pair is so small that drawings are likely to be less accurate. Standard deviations of Pairs II to VI, as percentages of TCL, show a slight downward trend with increased length. It has been decided to make a more extensive statistical analysis of the original data and the results will be published later.

*Hylemya cilicrura* (Rond.) *and Hylemya trichodactyla* (Rond.)

According to Brooks (4) "These species can be distinguished from each other for certain only in the adult male". Under these circumstances one must either rear the progeny of definitely identified adults for study or examine mixed collections looking for a clue to their separation. Only the latter course was available for this study and, accordingly, complements of some larvae from collections taken at Brandon, Man., Cloverdale, B.C., Belleville, Ont., and Guelph, Ont. were studied and other larvae from these same collections were reared for identification. The adults were identified by Mr. J. F. McAlpine and his determinations have been recorded in footnotes to Table III. Fortunately, only *H. cilicrura* was found in the Brandon sample but both *H. cilicrura* and *H. trichodactyla* were reared from the sample from Cloverdale. The Brandon collection was, therefore, used in describing the chromosomes of *H. cilicrura*, and 20 complements from four Cloverdale larvae in which the chromosomes differed distinctly and uniformly from those of the Brandon complements have been used in describing what is probably the complement of *H. trichodactyla*. Results from the other collections will also be compared.

Complements were examined from 26 Brandon larvae (*H. cilicrura*). Of these larvae, 20 had the usual complement of 12 chromosomes but the others had from 11 to 15 chromosomes in different larvae. The TCL is based on the normal 12 chromosomes only; the extra ones will be referred to as supernumeraries (*S*) and discussed in more detail later. Results of analysis of the 29 complements studied will be found in Table VII and Text-fig. 4, and

photomicrographs of a variety of different metaphases constitute Plate I, Figs. 3 to 6 inclusive. A high proportion of complements with supernumeraries have been included in the 29 but since their analyses seem to be quite similar to the others, it is unlikely that their inclusion produces a distorted average result.

The 29 complements ranged from 52.4 $\mu$ to 112.7 $\mu$ in TCL, with 78.0 $\mu$ as the average. The acrocentric presumed sex pair averaged 4.4% of the TCL and the average of the arm ratios of Pairs II–VI inclusive was 1.70. The arm ratios of adjacent pairs in Pairs II–VI inclusive do not overlap. Pairs II and III overlap slightly in length but Pair VI is quite distinct. These circumstances, in combination with the distinct secondary constrictions in the short arms of Pair III chromosomes (see later), enable one to identify the members of each pair reasonably accurately.

Metaphases from five Cloverdale larvae were studied. All had the usual 12 chromosomes consisting of a small acrocentric pair and five larger submetacentric pairs. Analysis revealed that the complements of four of these larvae (larvae 1–4 in Table II and Cloverdale *A* in Table III) were different from those of the fifth (larva 5 in Table II and Cloverdale *B* in Table III) which closely resembled complements from the *H. cilicrura* larvae from Brandon. Twenty-two adults from the Cloverdale collection have been identified as 17 *H. cilicrura* and five *H. trichodactyla*. Although this proportion is the reverse of that for the small sample of larvae, it does seem that four of the five larvae were nevertheless *H. trichodactyla*. Of course, other explanations are possible but results of analysis for the one Cloverdale larva do resemble those for larvae from Brandon and accordingly the four are considered likely to be *H. trichodactyla*.

The 20 complements from these four larvae ranged in TCL from 58.2 $\mu$ to 83.3 $\mu$ and averaged 68.5 $\mu$. The results of analysis are shown in Table II and the complement is illustrated in Plate I, Fig. 12. Pairs III and IV both

TABLE II

*H. trichodactyla* AND *H. cilicrura*, ANALYSES OF COMPLEMENTS FROM CLOVERDALE

| | Chromosome pair | | | | | |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI |
| Arm ratio | | | | | | |
| Av. larvae 1–4 | — | 1.45 ± 0.120 | 2.35 ± 0.151 | 1.37 ± 0.104 | 1.77 ± 0.167 | 1.40 ± 0.072 |
| Av. larva 5 | — | 1.38 ± 0.140 | 2.49 ± 0.113 | 1.46 ± 0.037 | 2.06 ± 0.153 | 1.39 ± 0.051 |
| Percentage of TCL | | | | | | |
| Av. larvae 1–4 | 3.8 ± 0.528 | 16.5 ± 0.560 | 18.0 ± 0.401 | 18.0 ± 0.516 | 20.4 ± 0.620 | 23.3 ± 0.696 |
| Av. larva 5 | 3.7 ± 0.600 | 16.6 ± 0.707 | 18.4 ± 0.529 | 19.1 ± 0.592 | 19.3 ± 0.648 | 22.8 ± 1.288 |

*Average TCL for 20 complements in larvae 1–4 was 68.5 $\mu$; for 3 in larva 5, 68.7 $\mu$.*

average 18.0% of the TCL but they differ greatly in their arm ratios. All other adjacent pairs are either quite distinct or nearly so in both arm ratios and percentages of TCL and, accordingly, can be identified with accuracy. The average of arm ratios for Pairs II to VI inclusive is 1.67.

These Cloverdale complements differ from those of Brandon larvae in several respects. Pair IV constitutes 18.0% of the TCL here but 19.1% in the Brandon larvae. Pair V has a lower arm ratio here and averages 20.4% of the TCL as against 19.2% for Brandon. Brandon Pairs IV and V are very similar in size, whereas Cloverdale Pair V is consistently larger than Pair IV. Pair I is also smaller, though this is probably not significant since there is considerable variation in length. The complements from these two sources were examined for possible differences in secondary arm ratios with the following results:

| | IIL* | IIS* | IIIL | IIIS | IVL | IVS | VL | VS | VIL | VIS |
|---|---|---|---|---|---|---|---|---|---|---|
| Brandon | 0.87(2) | 1.03(5) | 0.51(4) | 0.90(38) | 3.70(2) | 3.26(6) | 0.54(18) | 0.99(7) | 3.69(15) | 3.52(18) |
| | | 1.93(2) | 0.95(3) | | 0.22(1) | 1.43(1) | 1.06(3) | 1.62(7) | 0.24(2) | 1.20(1) |
| | | | 5.03(2) | | | 0.44(1) | 2.80(1) | | 1.84(2) | |
| Cloverdale *A* | 0.64(1) | 0.72(5) | 1.20(18) | 0.63(33) | 2.72(2) | 4.83(2) | 0.87(5) | 1.45(2) | 3.34(8) | 3.74(26) |
| | 2.50(1) | 1.79(3) | | | 0.58(2) | 0.67(3) | 0.18(1) | 3.00(1) | 1.74(3) | 1.87(1) |
| | 4.50(1) | | | | 1.31(1) | | 4.00(1) | 0.33(1) | 1.00(1) | |
| Cloverdale *B* | — | 0.87(2) | — | 0.77(6) | — | — | 0.56(4) | 1.00(1) | — | 4.00(1) |

* L = *long arm*, S = *short arm*.

Numbers in parentheses are the numbers of chromosomes used to calculate the given average. The secondary constrictions in the short arms of Pair III are particularly distinct and indicate a possible difference. Other differences are apparent and though they are less convincing, they support the concept of dissimilarity of the complements from the two sources.

The larvae from Guelph and Charlottetown were selected on morphological grounds by Mr. Wishart as being "possibly *H. trichodactyla*". Larvae from Belleville were tentatively considered to be *H. cilicrura*. The averages for complements of these larvae will be found in Table III. Pair III of Belleville larvae has an unusually high arm ratio and Guelph larvae are similar in this respect. The two complements from P. E. I. differ from the others in certain respects but these differences are of questionable significance also. It is, of course, possible that local populations of *H. cilicrura*, differing in certain chromosomes, exist.

The presumed sex chromosomes and the supernumeraries in *H. cilicrura* merit separate consideration. The two chromosomes of Pair I differ slightly in length in certain larvae having the usual complement, though both have

TABLE III

COMPARISON OF COMPLEMENTS FROM DIFFERENT COLLECTIONS OF *H. cilicrura* AND *H. trichodactyla*

| Source | Chromosome pair | | | | | | | | | | | No. of cells | No. of larvae | Average TCL in $\mu$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | | III | | IV | | V | | VI | | | | |
| | % TCL | Arm ratio | % TCL | Arm ratio | % TCL | Arm ratio | % TCL | Arm ratio | % TCL | Arm ratio | % TCL | | | |
| Brandon** | 4.4 | 1.38 | 16.4 | 2.35 | 18.3 | 1.36 | 19.1 | 2.04 | 19.2 | 1.39 | 22.5 | 29 | 9 | 78.0 |
| Cloverdale *A** | 3.8 | 1.45 | 16.5 | 2.35 | 18.0 | 1.37 | 18.0 | 1.77 | 20.4 | 1.40 | 23.3 | 20 | 4 | 68.5 |
| Cloverdale *B*** | 3.7 | 1.38 | 16.6 | 2.49 | 18.4 | 1.46 | 19.1 | 2.06 | 19.3 | 1.39 | 22.8 | 3 | 1 | 68.7 |
| Belleville** | 4.3 | 1.38 | 16.4 | 2.59 | 18.2 | 1.40 | 19.6 | 2.03 | 19.3 | 1.36 | 22.3 | 20 | 7 | 71.8 |
| Guelph** | 3.8 | 1.36 | 16.8 | 2.52 | 18.1 | 1.38 | 19.4 | 2.05 | 19.2 | 1.43 | 22.6 | 8 | 3 | 75.4 |
| Charlottetown | 3.8 | 1.24 | 17.7 | 2.35 | 17.3 | 1.36 | 19.5 | 1.67 | 19.7 | 1.40 | 22.1 | 2 | 1 | 83.2 |

** Considered to be H. trichodactyla, see text.*
*** Considered to be H. cilicrura, see text.*
*Note re adults reared from these collections:*
*Brandon, 13 adults, all H. cilicrura.*
*Cloverdale, 22 adults = 17 H. cilicrura and 5 H. trichodactyla.*
*Guelph, 2 adults, both H. trichodactyla.*

subterminal secondary constrictions. The longer has been considered for reasons mentioned previously to be the *Y*-chromosome. One out of 26 larvae had only one sex chromosome, somewhat larger than usual. Four others had a 13th chromosome which ranged, in different individuals, from very small (Plate I, Fig. 6) to large and conspicuous, consisting of three or four segments (see Plate I, Fig. 4). One larva had three very small supernumeraries (Plate I, Fig. 5). The sex of the larvae carrying these extra chromosomes was not known, but, since only five of the 26 larvae examined had extra chromosomes and these five varied in the number and morphology of the extra chromosomes, it seems preferable to consider the extras as supernumeraries, rather than to consider that a compound sex-chromosome mechanism exists in the species *H. cilicrura*.

*Hylemya cana* Macquart

A microscope preparation labelled *H. cana* was received during the summer of 1951 from Mr. H. Bruneau. Since Brooks (4) has considered *H. cana* as synonymous with *H. cilicrura*, it is of interest to compare results of chromosome analyses. In this specimen the complements were small, ranging from 33.6 $\mu$ to 59.1 $\mu$ and averaging 44.0 $\mu$. A small chromosome, presumably supernumerary, about the same size as the Pair I chromosomes, was present with the usual six pairs. Five complements gave results as shown in Table VII. Except for the small size of Pair V and the high ratio for Pair III, the results are fairly close to those for *H. cilicrura* from Brandon. A decision regarding the significance, if any, of these differences must await further study.

*Hylemya brassicae* (Bouché)

Collections of this species were obtained, as shown in Table I, from a number of selected geographic locations and from several different host plants. The analyses of complements from larvae collected at Prestwick, Scotland, will be used as the basis for a general description of the complement of the species. This general description will be followed by a comparative study of complements from different sources.

Twenty metaphases from seven Prestwick larvae were analyzed. Twelve chromosomes, consisting of a small pair of presumed sex chromosomes and five pairs of submetacentric autosomes, were found in all complements; in fact no deviation in this respect was found in any complement from any of the samples of this species. The 20 complements averaged 61.7 $\mu$ in TCL with the highest 71.9 $\mu$ and the lowest 51.1 $\mu$. The details of analyses have been summarized in Table VII and are graphically represented in Text-fig. 5. The complement is essentially the same as that in a Belleville larva from radish (Plate I, Fig. 2).

The pairs in these 20 complements are fairly distinct. The acrocentric presumed sex pair is very small (3.4% of TCL) but, although one chromosome of this pair sometimes has a medial secondary constriction whereas the other sometimes has a subterminal one, the writer is still not satisfied that the *X*- and *Y*-chromosomes are distinguishable. The arm ratios of Pairs II and

III, though differing on the average by 0.25, overlap only slightly whereas their lengths overlap more extensively. All the other pairs have distinct ranges in their arm ratios and Pairs V and VI also have distinct length ranges. Within the individual complements no difficulties were encountered in this collection, though some pairs were not as definitely recognizable in a few complements from other collections.

Secondary constrictions were found in the arms of certain chromosomes of all pairs (see Table V) but only those more frequently recorded merit specific mention here. In some cases the short arm of Pair II has a secondary constriction which produces proximal segments averaging 1.21 times the distal ones in length, whereas corresponding proximal segments in the short arms of Pair III average only 0.87 of the distal ones. Similarly the short arms of Pair IV have, in a few cases, proximal segments which average 0.97 of the distal ones, whereas the short arms of Pair V are divided, in a majority of cases, to produce relatively shorter proximal segments (0.60 of distal) as compared to the distal ones. Pair VI chromosomes have subterminal secondary constrictions in a few chromosomes, which give average proximal : distal ratios of 4.02 for long arms and 3.75 for short ones. These constrictions help considerably in distinguishing Pair II from III, IV from V, and V from VI.

The averages derived from analyses of complements from different sources will be found in Table IV, in which corresponding results for *H. planipalpus* have been included for comparison. Despite the relatively high arm ratio for Pair IV and the shortness of Pair I, the four Cloverdale larvae may all be *H. brassicae* since three-quarters of adults reared from the collection were identified as such (see footnote Table I) and since these larvae were collected together, whereas *H. planipalpus* larvae are usually collected singly. They are sufficiently different to suggest that a separate group of *H. brassicae* (or of *H. planipalpus*) exists in the Pacific coast region. (This group could belong to the recently described species, *H. pseudobrassicae*.) The *H. planipalpus* collection has a very low arm ratio for Pair IV (av. 2.62) and the Guelph collection, which is very high (av. 3.10) in this respect, has a low arm ratio for Pair V (av. 2.11) and Pair I seems unusually long. The latter collection differs consistently from other *brassicae* collections and it may be that it represents a specialized group in the species rather than merely a sample with more than usual chance variation from the average for the species.

An attempt was made to compare the positions of secondary constrictions in complements from the different collections (Table V). Although certain constrictions are rarely observed, and may be nothing more than artifacts produced by the squashing technique, some of them are so regularly detected in the same region that they must certainly be characteristic of the chromosomes possessing them. However, the variations in the averages of the secondary arm ratios obtained from small numbers of chromosomes for the different collections may be considered of doubtful importance, though the differences between chromosome pairs and between different collections should be of some value where a sufficient number of observations is available.

TABLE IV

*H. brassicae* AND *H. planipalpus*, SOMATIC CHROMOSOME COMPLEMENTS OF DIFFERENT COLLECTIONS

| Collection | Chromosome pair | | | | | | | | | | | Av. TCL in $\mu$ | No. cells analyzed | No. of larvae |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | | II | | III | IV | | V | | VI | | | | |
| | % TCL | Arm ratio | % TCL | Arm ratio | % TCL | Arm ratio | % TCL | Arm ratio | % TCL | Arm ratio | % TCL | | | |
| *H. brassicae* | | | | | | | | | | | | | | |
| (1) Belleville radish | 3.39 | 2.13 | 16.7 | 2.36 | 17.6 | 2.75 | 18.8 | 2.17 | 19.8 | 1.46 | 23.8 | 80.0 | 13 | 8 |
| (2) Belleville cabbage | 3.33 | 2.01 | 16.5 | 2.35 | 17.8 | 2.83 | 18.7 | 2.31 | 19.9 | 1.47 | 23.8 | 73.5 | 20 | 11 |
| (3) Cloverdale* radish | 3.07 | 2.03 | 16.7 | 2.40 | 18.1 | 2.98 | 18.9 | 2.34 | 19.6 | 1.51 | 23.6 | 63.8 | 10 | 4 |
| (4) Charlottetown turnip | 3.49 | 2.13 | 16.7 | 2.42 | 17.8 | 2.85 | 18.3 | 2.21 | 20.0 | 1.53 | 23.7 | 66.3 | 11 | 5 |
| (5) Guelph turnip | 3.52 | 2.03 | 16.9 | 2.41 | 17.8 | 3.10 | 18.3 | 2.11 | 19.8 | 1.55 | 23.7 | 71.1 | 25 | 10 |
| (6) Prestwick br. sprouts | 3.38 | 2.05 | 17.1 | 2.30 | 17.9 | 2.85 | 18.3 | 2.18 | 19.9 | 1.52 | 23.5 | 61.7 | 20 | 7 |
| (7) St. John turnip | 3.25 | 2.11 | 16.9 | 2.31 | 18.1 | 2.84 | 18.7 | 2.15 | 19.7 | 1.50 | 23.5 | 71.5 | 20 | 9 |
| *H. planipalpus* | 3.47 | 2.07 | 16.2 | 2.33 | 17.8 | 2.63 | 18.2 | 2.28 | 20.7 | 1.58 | 23.7 | 66.6 | 20 | 8 |

* *Possibly H. pseudobrassicae, see text and Table I footnote.*

TABLE V

*H. brassicae* AND *H. planipalpus*, SECONDARY RATIOS IN THE ARMS OF CHROMOSOMES OF DIFFERENT COLLECTIONS

| Collections | Chromosome pair | | | | | | | | | | Total no. chrom. of each pair |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | II | | III | | IV | | V | | VI | | |
| | L. arm | S. arm | L. arm | S. arm | L. arm | S. arm | L. arm | S. arm | L. arm | S. arm | |
| *H. brassicae* | | | | | | | | | | | |
| (1)** | 0.98 (7)* | 1.06 (4)<br>0.50 (2) | 0.63 (5) | 0.82 (7) | 0.89 (5)<br>1.15 (4) | 0.87 (5) | 1.01 (4) | 0.49 (13) | 3.85 (5)<br>1.16 (4) | 4.14 (16) | 26 |
| (2) | 0.72 (4)<br>5.50 (1)<br>0.18 (1) | 1.10 (16)<br>0.40 (1) | 0.76 (6)<br>2.39 (5)<br>4.80 (1) | 0.91 (10) | 1.60 (5)<br>0.21 (3)<br>3.50 (1) | 0.86 (9)<br>1.84 (1) | 1.64 (5)<br>0.66 (1)<br>0.20 (1) | 0.58 (17)<br>3.00 (1) | 4.76 (7)<br>1.05 (5)<br>0.17 (1) | 3.41 (14)<br>0.43 (1) | 40 |
| (3) | 1.40 (1) | 1.36 (4) | 3.42 (2) | — | 6.50 (1) | 0.86 (2) | — | 0.52 (15) | 5.57 (1)<br>0.35 (1) | 3.69 (6) | 20 |
| (4) | 0.92 (5) | 1.13 (15) | 0.77 (2) | 1.06 (4) | 0.70 (2)<br>4.20 (2) | 1.11 (11) | 1.04 (3)<br>0.48 (1) | 0.49 (16) | 3.40 (5) | 3.93 (9)<br>0.70 (1) | 22 |
| (5) | 1.15 (4) | 0.99 (16)<br>0.50 (1) | 2.48 (5)<br>0.28 (4) | 1.13 (16) | 0.57 (3)<br>3.04 (3) | 1.19 (11) | 3.28 (3) | 0.54 (34) | 3.76 (9)<br>1.57 (5)<br>0.29 (5) | 3.85 (12)<br>1.34 (2) | 50 |
| (6) | 0.84 (4)<br>0.38 (1) | 1.21 (7) | 3.80 (1) | 0.87 (10) | 1.02 (4) | 0.97 (7) | 0.75 (1)<br>1.16 (1) | 0.60 (25) | 4.02 (5)<br>0.28 (1) | 3.75 (7) | 40 |
| (7) | 0.60 (1)<br>1.72 (1)<br>0.24 (1) | 1.00 (10)<br>2.00 (1)<br>0.45 (1) | 0.95 (4)<br>2.82 (2)<br>6.00 (1) | 1.06 (5)<br>2.08 (3) | 0.67 (4)<br>2.77 (2) | 0.86 (3) | 0.63 (1)<br>1.83 (1)<br>0.24 (1) | 0.52 (24) | 3.77 (8)<br>1.82 (1)<br>0.80 (1) | 4.20 (19)<br>1.16 (1) | 34 |
| *H. planipalpus* | | | | | | | | | | | |
| | 1.05 (6)<br>3.16 (1) | 0.78 (10) | 2.19 (2)<br>6.00 (2)<br>0.62 (1) | 0.89 (5) | 1.47 (2)<br>0.16 (1) | 0.90 (10) | — | 0.55 (32) | 4.17 (8)<br>0.28 (1) | 4.08 (14)<br>1.86 (1) | 40 |

* *Figures in parentheses = number of chromosomes used to obtain averages, etc.*
** *Collections numbered as in Table IV.*
*Note: L. arm = long arm and S. arm = short arm.*

*Hylemya planipalpus* (Stein)

The eight larvae used for analyses of this species were collected at Brandon. All larvae had 12 chromosomes, closely resembling those of *H. brassicae* and ranging in TCL from 48.8 $\mu$ to 86.3 $\mu$ with 66.6 $\mu$ as the average. Results of analysis have been recorded in Table VII, and the complement is illustrated in Text-fig. 6 and Plate I, Fig. 11. The average results have been tabulated with those of different collections of *H. brassicae* in Tables IV and V for direct comparison.

The pairs are not quite so easy to identify as in *H. brassicae.* Pair I is very small so that it is not surprising that the *X*- and *Y*-chromosomes were not distinguishable. Pair II is nearly distinct from Pair III in percentage of TCL. Pairs III and IV differ little as percentages of the TCL though they have more distinct arm ratios. Pairs IV and V differ appreciably in average arm ratio (IV=2.63, V=2.28), and as percentages of the TCL they do not overlap. Pairs V and VI are distinctly different both in arm ratio and length.

Some supplementary information of help in identifying the pairs was derived from a study of the position of the secondary constrictions (see Table V). For example, the short arms of Pair V have each a distinct secondary constriction, observable in nearly all cells, which produces proximal segments 0.55 of the distal in length. The positions of these secondary constrictions resemble those of *H. brassicae.*

*Hylemya crucifera* Huck.

Collections of larvae of this species were obtained from Brandon and Saskatoon. The results for these collections will be compared following the description based on analyses of the 20 complements from 10 Brandon larvae. All these 20 complements contained 12 chromosomes each and ranged from 49.4 $\mu$ to 73.2 $\mu$ with an average of 59.2 $\mu$ for the TCL. The results of analysis have been arranged in Table VII and the complement illustrated in Text-fig. 7 and Plate I, Fig. 7.

The presumed sex Pair I averaged 4.33% of the TCL and the secondary constrictions, though somewhat variable in position, usually subdivided each chromosome into a proximal segment about half as long as the distal one. The *X*- and *Y*-chromosomes were not distinguishable. Arm ratios of Pairs II, III, IV, and V are not as distinct as in other species in this genus and percentages of TCL for these pairs differ only moderately so that considerable overlapping exists. The distinct secondary constriction in the short arms of Pair V (see later) assures ready identification. Pair VI is distinct in both size and arm ratio from Pairs III, IV, and V and in size from Pair II. The arm ratios of Pairs II–VI inclusive averaged 2.05.

Secondary constrictions were scarce, except in the short arms of Pair V, and generally irregular in position. One was located at the mid-point of the long arm of Pair IV in three cases and in the same position of the long arm of Pair V in three cases. In the short arm of Pair V, a distinct secondary constriction regularly provides a proximal segment averaging 0.49 of the

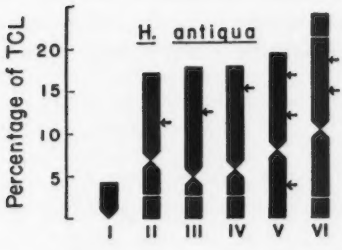



Figure 3

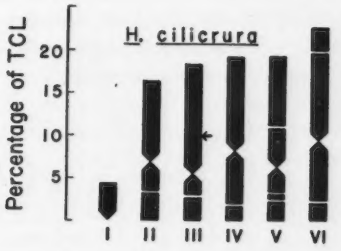



Figure 4

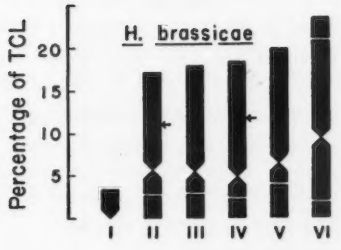



Figure 5

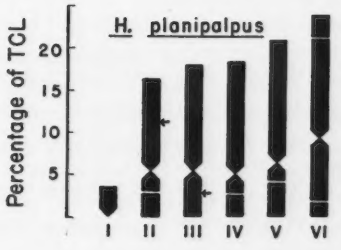



Figure 6

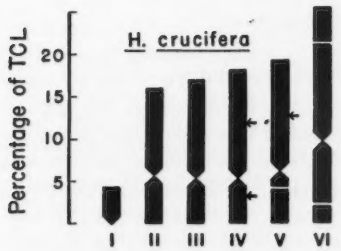



Figure 7

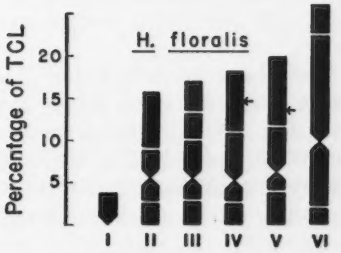



Figure 8

TEXT-FIGS. 3–8. Idiograms of the somatic chromosome complements of species of *Hylemya*. See text for explanation.

distal segment. Seven Pair VI chromosomes had their long arms divided by secondary constrictions to produce proximal segments averaging 2.71 times the distal ones in length and in four cases the short arms of this pair were similarly divided so that their proximal segments averaged 3.60 times the length of the distal segments. Other secondary constrictions were too infrequent to merit mention.

TABLE VI

COMPARISON OF COLLECTIONS OF *H. crucifera* AND *H. floralis*

| Chromosome pair | Arm ratios | | | Percentages of TCL | | |
|---|---|---|---|---|---|---|
| | *H. crucifera* | | *H. floralis* | *H. crucifera* | | *H. floralis* |
| | Brandon* | Saskatoon** | Elgin*** | Brandon* | Saskatoon** | Elgin*** |
| I | — | — | — | 4.3 ± 0.366 | 4.4 ± 0.385 | 3.8 ± 0.297 |
| II | 1.87 ± 0.185 | 1.85 ± 0.109 | 1.86 ± 0.115 | 16.0 ± 0.547 | 15.6 ± 0.494 | 15.7 ± 1.065 |
| III | 2.19 ± 0.157 | 2.16 ± 0.130 | 2.06 ± 0.104 | 17.0 ± 0.744 | 16.9 ± 0.732 | 16.8 ± 0.509 |
| IV | 2.33 ± 0.174 | 2.45 ± 0.128 | 2.47 ± 0.132 | 18.1 ± 0.615 | 18.0 ± 0.788 | 18.1 ± 0.541 |
| V | 2.16 ± 0.138 | 2.09 ± 0.156 | 2.19 ± 0.136 | 19.2 ± 0.685 | 19.4 ± 0.692 | 19.9 ± 0.640 |
| VI | 1.64 ± 0.064 | 1.65 ± 0.078 | 1.64 ± 0.072 | 25.4 ± 1.092 | 25.7 ± 0.995 | 25.8 ± 0.930 |
| Av. II–VI inclusive | 2.05 | 2.03 | 2.04 | — | — | — |
| Av. TCL in $\mu$ | — | — | — | 59.2 | 61.8 | 63.5 |

* *Twenty complements from 10 larvae from turnips.*
** *Twenty complements from nine larvae from turnips.*
*** *Twenty-one complements from 10 larvae.*
*Note: numbers following averages are standard deviations.*

It will be noted from Table VI that the results of analysis of the 20 complements from Saskatoon larvae agree very well with those of the Brandon collection. No differences were found in position of secondary constrictions. One must conclude that there is no evidence of morphological differences between the chromosomes of these two collections. Differences from those of *H. floralis* will be considered later.

*Hylemya floralis* (Fall.)

The larvae used for the study of this species were collected at Elgin, Scotland. The host plant was not specified. All of the 21 complements analyzed consisted of 12 chromosomes. These complements ranged from 49.7 $\mu$ to 90.8 $\mu$ with an average of 63.5 $\mu$. The results of analysis have been recorded in Table VII and the complement illustrated in Text-fig. 8 and Plate I, Fig. 8.

The presumed sex Pair I averaged 3.76 as percentage of the TCL and a secondary constriction was found rarely and at inconsistent positions. This pair is clearly recognizable because of its small size but the *X* and *Y* are indistinguishable. Pairs II and III overlap appreciably as percentages of the TCL and, since their arm ratios are only moderately different, they overlap to some extent in this respect also. Within a particular complement, however, they differ in one respect or the other so that real difficulty in distinction is rarely encountered. Pairs III and IV are quite distinct in their arm ratios and also differ appreciably in length. Both arm ratios and lengths differ between Pairs IV and V, though both overlap somewhat. Pairs V and VI are quite distinct in both respects. Arm ratios of Pairs II–VI inclusive averaged 2.04.

Secondary constrictions were found in many of the chromosomes of this species. Fourteen of the 42 chromosomes of Pair II had a secondary constriction in the long arm which produced a proximal segment averaging 0.53 of the distal segment, whereas 20 of the short arms of Pair II were similarly divided to produce proximal segments 1.12 times the distal segment in length. A quarter of the Pair III chromosomes either possessed a secondary constriction in the long arm, giving an average proximal segment 0.63 of the distal in length, or had the short arm divided into two almost equal segments. Similarly 12 chromosomes of Pair IV had their long arms divided to produce proximal segments averaging 0.83 of the distal segments and 12 had the short arms divided into two nearly equal segments. In eight cases a secondary constriction was noticed in the long arm of chromosomes of Pair V near the kinetochore but a constriction in the short arm was conspicuous in a majority of cases and produced a proximal segment averaging 0.59 of the distal. Many Pair VI chromosomes had secondary constrictions producing proximal: distal ratios averaging 3.53 in the long arms and 3.57 in the short arms. The application of these additional means of identifying the chromosomes removed most of the difficulties in analysis of these complements; in fact, analysis was relatively easy.

*Hylemya fugax* (Mg.)

The interesting complement of this species has been described in a general way elsewhere (1). The details of analyses have not been recorded previously and will be presented here.

In this species males have 13 chromosomes and females 14. The male complement consists of five submetacentric pairs of autosomes, a large sex chromosome, $X_1$, a *Y*-chromosome somewhat smaller than $X_1$, and a still smaller $X_2$ chromosome. Females have five submetacentric pairs of autosomes plus two $X_1$ and two $X_2$ sex chromosomes. It is, of course, necessary to use twice the length of the $X_1$ and $X_2$ instead of the combined length of $X_1$, $X_2$, and *Y* when determining the TCL of male complements in order to make them comparable to those from females.

The 22 metaphases analyzed came from 10 larvae collected at Wye, England. The complements averaged 100.4 $\mu$ in their TCL with a range from 80.8 $\mu$ to 128.4 $\mu$. The results of analyses have been recorded in Table VII and complements are illustrated in Text-fig. 9 and Plate I, Figs. 9 and 10.

The three types of sex chromosomes differ strikingly from each other. The small acrocentric $X_2$ chromosomes (Pair $I_2$) constitute 2.9% of the TCL and are not noticeably heterochromatic at metaphase. Sometimes their chromatids are widely separated except at the kinetochore, giving the impression that each chromosome has a medial constriction. At other times there appears to be a true secondary medial constriction. The large acrocentric $X_1$ (Pair $I_1$) constitutes 10.0% of the TCL; it is not noticeably heterochromatic at metaphase, and has two secondary constrictions. The positively heteropycnotic and metacentric *Y*-chromosome is intermediate in size between the $X_1$ and the $X_2$ and averages 7.3% of the TCL for the seven male complements analyzed. It has an arm ratio of 1.79 for these seven cases. It will be obvious that the presence of these unusual sex chromosomes constitutes an important characteristic of the complement of this species.

The five autosomal pairs are distinctly larger than the sex chromosomes. The arm ratios of Pairs II and III overlap but their percentages of TCL do not. The short arms of Pair II are distinctly heterochromatic. Pairs III and IV are similar in their average arm ratios and differ by only 1.2 in average percentage of the TCL. Pairs IV and V have quite different arm ratios with only slight overlapping, but they overlap appreciably in length. Pair V is distinct in length but not in arm ratio. The average of arm ratios of Pairs II–VI inclusive is 1.49.

Fortunately secondary constrictions are frequent and in several cases quite distinct. The long acrocentric $X_1$ has a short proximal segment separated from the distal segment, which averages 12 times as long, by a distinct secondary constriction. (These segments differ more in size in somatic cells than they do during meiosis.) Another secondary constriction divides the distal segment into nearly equal parts, the more proximal part averaging 1.38

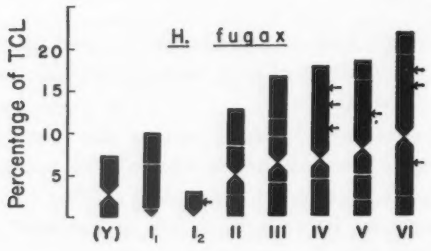



Figure 9

TEXT-FIG. 9. Idiogram of the somatic chromosome complement of *Hylemya fugax*. See text for explanation.

times the distal for the seven $X_1$ chromosomes in which this constriction was seen. Pair II has a secondary constriction in the long arm, which for 18 chromosomes gave a proximal segment averaging 0.73 times the distal one whereas seven short arms had proximal segments averaging 1.15 times a distal one. Twenty-six secondary constrictions found in the long arms of Pair III varied in distinctness and location but definite secondary constrictions were regularly found in the short arms providing proximal segments 0.57 times the distal in average length. Short arms of Pair IV were distinctly divided into two segments with the proximal 0.54 of the distal one, but constrictions in the long arms were variable in location. The distal segment of the short arm has a smaller proximal part (about one third of the distal part in length) distinctly recognizable in 12 cases. Subterminal and submedial secondary constrictions were seen in both long and short arms of Pair V but measurements of the location of these constrictions proved so variable that averages give a poor indication of their true position. In Pair VI the constrictions are somewhat more uniform in location giving proximal to distal segment ratios of 3.57 for 16 long arms and 3.04 for 32 short arms. The value of these constrictions in assigning chromosomes to their proper pair will be obvious.

## General Discussion and Conclusions

The *Hylemya* species studied may be arranged, in a general way, as follows:

| | |
|---|---|
| I. Twelve-chromosome species | |
| 1. Species having II–VI arm ratios averaging about 2.20 | *H. brassicae*<br>*H. planipalpus* |
| 2. Species having II–VI arm ratios averaging about 2.05 | *H. crucifera*<br>*H. floralis* |
| 3. Species having II–VI arm ratios averaging about 1.65–1.80 | *H. antiqua*<br>*H. cilicrura*<br>*H. trichodactyla* ?<br>*H. cana* |
| II. Thirteen/fourteen-chromosome species | |
| 1. Species having II–VI arm ratios averaging about 1.5 | *H. fugax* |

These categories differ in other respects than those listed above as will be apparent from a close examination of Text-figs. 3–9 inclusive, tables presented earlier in this publication, and Table VII. For example, note arm ratios for Pair II average about 2.06 for group I—1, about 1.86 for group I—2, and about 1.45 for group I—3. Accordingly, these groups seem quite distinct as presented, though future additions of other species may complicate the picture.

TABLE VII

COMPARISON OF SOMATIC COMPLEMENTS OF *Hylemya* SPECIES

| Species | Chromosome pair | | | | | | Av. | No. comp. analyzed |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | | |
| *Arm ratios* | | | | | | | | |
| *H. brassicae* (Prestwick) | — | 2.05 ± 0.131 | 2.30 ± 0.097 | 2.85 ± 0.150 | 2.18 ± 0.179 | 1.52 ± 0.070 | 2.18 | 20 |
| *H. planipalpus* | — | 2.07 ± 0.097 | 2.33 ± 0.164 | 2.63 ± 0.164 | 2.28 ± 0.130 | 1.58 ± 0.062 | 2.18 | 20 |
| *H. crucifera* (Brandon) | — | 1.87 ± 0.185 | 2.19 ± 0.157 | 2.33 ± 0.174 | 2.16 ± 0.138 | 1.64 ± 0.064 | 2.05 | 20 |
| *H. floralis* | — | 1.86 ± 0.115 | 2.06 ± 0.104 | 2.47 ± 0.132 | 2.19 ± 0.136 | 1.64 ± 0.072 | 2.04 | 21 |
| *H. antiqua* | — | 1.50 ± 0.108 | 2.59 ± 0.169 | 2.08 ± 0.157 | 1.45 ± 0.108 | 1.30 ± 0.076 | 1.78 | 80 |
| *H. cilicrura* (Brandon) | — | 1.38 ± 0.084 | 2.34 ± 0.162 | 1.36 ± 0.114 | 2.04 ± 0.162 | 1.39 ± 0.090 | 1.70 | 29 |
| *H. trichodactyla* ? (Cloverdale) | — | 1.45 ± 0.120 | 2.35 ± 0.151 | 1.37 ± 0.104 | 1.77 ± 0.167 | 1.40 ± 0.072 | 1.67 | 20 |
| *H. cana* | — | 1.35 ± 0.094 | 2.63 ± 0.063 | 1.34 ± 0.085 | 1.81 ± 0.109 | 1.38 ± 0.066 | 1.70 | 5 |
| *H. fugax* | — | 1.56 ± 0.167 | 1.65 ± 0.124 | 1.60 ± 0.131 | 1.30 ± 0.077 | 1.32 ± 0.093 | 1.49 | 22 |

TABLE VII—(*Concluded*)

COMPARISON OF SOMATIC COMPLEMENTS OF *Hylemya* SPECIES—(*Concluded*)

| Species | Chromosome pair | | | | | | Av. | No. comp. analyzed |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | | |
| *Percentages of TCL* | | | | | | | | |
| *H. brassicae* (Prestwick) | 3.4 ± 0.363 | 17.1 ± 0.647 | 17.9 ± 0.453 | 18.3 ± 0.695 | 19.9 ± 0.502 | 23.5 ± 0.855 | 61.7* | 20 |
| *H. planipalpus* | 3.5 ± 0.385 | 16.2 ± 0.627 | 17.8 ± 0.376 | 18.2 ± 0.554 | 20.7 ± 0.593 | 23.7 ± 0.778 | 66.6 | 20 |
| *H. crucifera* | 4.3 ± 0.366 | 16.0 ± 0.547 | 17.0 ± 0.744 | 18.1 ± 0.615 | 19.2 ± 0.685 | 25.4 ± 1.092 | 59.2 | 20 |
| *H. floralis* | 3.8 ± 0.297 | 15.7 ± 1.066 | 16.8 ± 0.509 | 18.1 ± 0.521 | 19.9 ± 0.640 | 25.8 ± 0.930 | 63.5 | 21 |
| *H. antiqua* | 4.3 ± 0.456 | 17.1 ± 0.614 | 17.6 ± 0.604 | 17.8 ± 0.598 | 19.3 ± 0.553 | 23.9 ± 0.720 | 85.4 | 80 |
| *H. cilicrura* (Brandon) | 4.4 ± 0.455 | 16.4 ± 0.390 | 18.3 ± 0.711 | 19.1 ± 0.788 | 19.2 ± 0.488 | 22.5 ± 0.792 | 78.0 | 29 |
| *H. trichodactyla* ? (Cloverdale) | 3.8 ± 0.528 | 16.5 ± 0.560 | 18.0 ± 0.401 | 18.0 ± 0.516 | 20.4 ± 0.620 | 23.3 ± 0.696 | 68.5 | 20 |
| *H. cana* | 4.3 ± 0.587 | 16.1 ± 1.204 | 18.4 ± 0.763 | 19.9 ± 0.409 | 18.9 ± 0.612 | 22.5 ± 0.947 | 44.0 | 5 |
| *H. fugax* | 12.9 ± 0.717 | 12.7 ± 0.478 | 16.6 ± 0.504 | 17.8 ± 0.508 | 18.4 ± 0.638 | 21.7 ± 0.701 | 100.4 | 22 |

* *Averages of TCL in μ. Note: figures following averages are standard deviations.*

The separation of species of *Hylemya* within the groups listed above on the basis of chromosomal differences constitutes a more difficult task. In several respects mean values of *planipalpus* chromosomes lie beyond the extremes of the ranges of corresponding values of collections of *brassicae*. The value of the differences as a means of separating these species can be determined by direct tests with known collections. It is possible that other populations of *brassicae* may yield statistics beyond the present ranges of variation, but present results indicate that the complement of *planipalpus* differs slightly from that of *brassicae*. Only minor differences exist between the complements of *crucifera* and *floralis*. The latter has a smaller sex pair and a slightly lower arm ratio for Pair III than the former. These differences seem sufficient to indicate that the two complements are dissimilar, but it may be that in interspecific hybrids meiotic disturbances resulting in sterility would not occur. (Nothing can be deduced regarding genetic differences between these species on the basis of the cytological observations presented in this paper.) Differences between complements of *antiqua* and *cilicrura*, although not great, involve all the pairs. Perhaps Pair V of *antiqua* should be compared with Pair IV of *cilicrura* but, though this would make the arm ratios more comparable, it would make the lengths less comparable. The similarity in the location of the secondary constriction in the short arm of Pair III in these two species (secondary ratio 0.90 in both) is impressive. Both *antiqua* and *trichodactyla* (?) differ from *cilicrura* in the relative lengths of Pairs IV and V and the two first mentioned differ greatly in the arm ratios of these pairs. These differences are more significant if the large number of complements involved is kept in mind. These considerations lead to the general conclusion that each of the *Hylemya* species studied has a characteristic complement of chromosomes which, by careful analysis, distinguishes it from other species.

Differences between complements of collections of a species from different locations apparently exist. For example, the larvae of the Guelph *H. brassicae* on turnip seem to have a slightly different complement than those from other localities, and the Belleville *H. cilicrura* (?) differs slightly from the Brandon collection, also on turnip, in the arm ratio of Pair III. These differences indicate the presence of intraspecific variation and, accordingly, merit further investigation to elucidate the extent and degree of the variation. On the other hand, the largest differences between complements of *H. brassicae* from radish and cabbage at Belleville were 0.14 for Pair V, and 0.12 for Pair II, in average arm ratio, with close agreement in nearly all other respects so that there is no satisfactory indication of any chromosome difference between these collections from different host plants. The intraspecific differences between samples from different host plants and geographic locations are generally less than the interspecific differences between the *Hylemya* species studied. However, a careful comparison indicates that two collections of *H. brassicae* had complements which differed nearly as much as the *H. planipalpus* complement differed from that of any collection of *H. brassicae*. Similarly the differences between complements of *H. floralis* and *H. crucifera* seem only slightly greater

than the differences between complements of the latter from Saskatoon and Brandon. Other examples might be quoted and it may be that intergeneric differences between complements in the Anthomyiidae will be found generally to exceed the intrageneric differences, as suggested later in this paper. The writer does not feel competent, however, to decide whether or not the extent of differences between the chromosome complements is proportional to the degree of morphological difference found between larvae or adults of taxonomic groups in the genus *Hylemya*, but present results suggest that this may hold for morphological differences of the larvae at least. Certainly, results herein support the validity of the present species classification in the genus *Hylemya*, except that the complement of *H. fugax* differs enough for this species to be placed in a separate genus, as some taxonomists have already done.

It should be interesting to compare the chromosomes of the *Hylemya* species studied with those of other genera in the Anthomyiidae. Measurements were attempted on Frolowa's (5) Fig. 12A of the complement of *Pegomyia geniculata*, which has six pairs of chromosomes as indicated previously (3), with the following results:

| | I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|
| Arm ratio | — | 1.92 | 2.00 | 2.12 | 2.00 | 1.27 |
| Percentage of TCL | 5.6 | 13.9 | 17.5 | 18.1 | 20.5 | 24.4 |

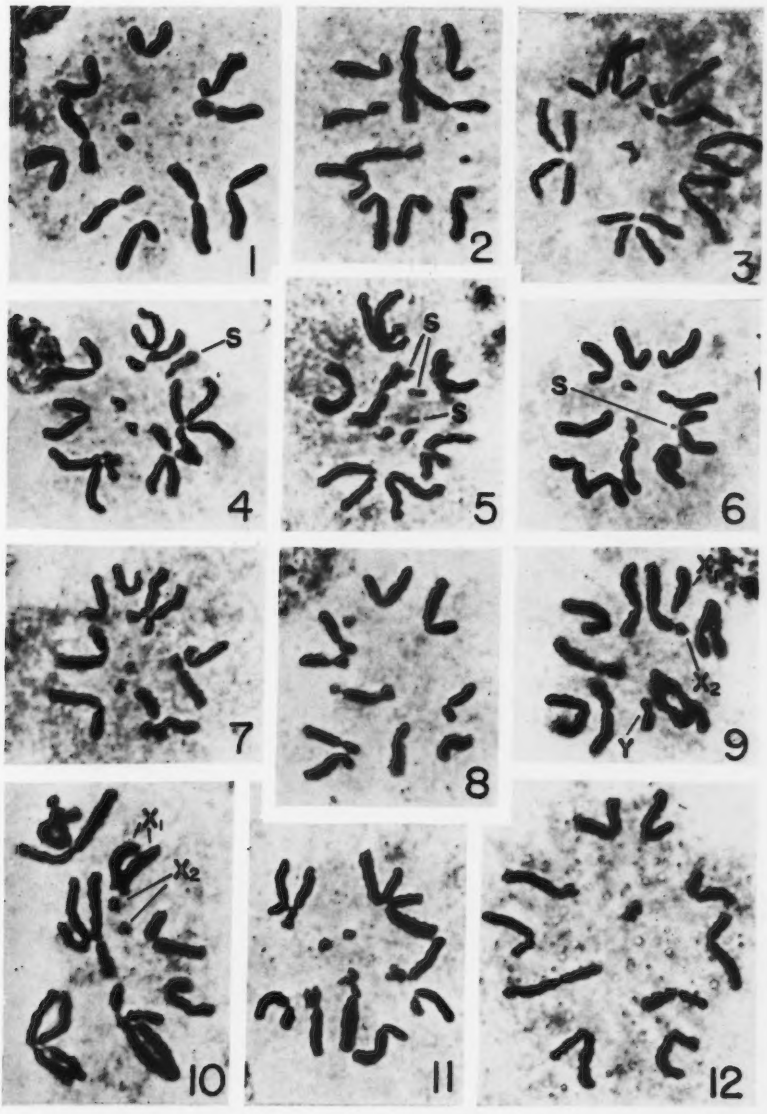
These figures bear some resemblance to those for *H. crucifera* and *H. floralis* but the sex chromosomes are longer, Pair II shorter, Pair IV has a lower arm ratio, etc. Stevens (7) has figured six pairs of chromosomes in stages of meiosis in *Phorbia brassica* but kinetochores are not shown and the only point of note is the marked difference in the heteromorphic pair (presumably sex chromosomes). Metz (6) described 12-chromosome complements in *Homalomya* sp., *Fucellia marina*, and *Ophyra leucostoma*. He stated "The chromosome group and pairing phenomena of Ophyra are practically the same as those of Homalomya and Fucellia." The complements figured resemble in general those of *Hylemya* species but, since the figures are unsuitable for measurement, detailed comparisons are not possible. It is noteworthy that his figures of the somatic complements of males of *Homalomya* sp. and *Ophyra leucostoma* do not show the sex pairs as heteromorphic whereas the sex pair of *Fucellia marina* is decidedly so. The writer (unpublished) has seen the somatic complement of *Fannia grisescens* (presumably female), which also consists of six pairs of chromosomes, but the homomorphic pair of sex chromosomes is comparable with the autosomes in size. Concerning comparisons of these complements with those of *Hylemya* species described in this paper, little can be said except that (*a*) the complements have the same number and general size relationships of the chromosomes and (*b*) the sex chromosomes in males of this family may be nearly homomorphic as in most of the *Hylemya* species described above, or strongly heteromorphic as seen in *Phorbia brassica* and *Fucellia marina*, or may constitute a sex trivalent as in *H. fugax*. Further investigations of this family should be very interesting, particularly regarding variations in the sex chromosomes.

The chromosomes of *H. fugax* provide material for study of a variety of interesting fundamental problems. They are much larger than those of *Drosophila melanogaster* so that pairing relations in both somatic pairing and meiosis can be studied with greater ease and accuracy. The trivalent $X_1X_2Y$ sex-chromosome mechanism provides material, with all members distinctly recognizable, for analysis of the behavior of such trivalents in higher Diptera. Also, chromosomes of Pair II, which have one arm heterochromatic and the other euchromatic, provide material for analysis of the differential behavior of these arms in somatic mitosis and in meiosis. Preliminary observations indicate a differential rate of contraction of the two arms in somatic prophase. However, such studies are beyond the scope of the present paper and will be discussed in a later report.

The discovery of supernumerary chromosomes in *H. cilicrura* is very interesting. We know that individuals of *H. fugax* have 13 (♂) or 14 (♀) chromosomes as compared with a basic 12 in all the other *Hylemya* species. Certainly some of the supernumeraries resemble the small sex chromosomes found in all the other *Hylemya* species investigated. At least one species, *Fucellia marina*, appears to have a long sex chromosome like the $X_1$ of *H. fugax* and one larva of *cilicrura* had a long supernumerary. It is possible that the *fugax* complement may have been derived from a complement carrying such a long supernumerary chromosome. The derivation might involve reciprocal translocations between the supernumerary and the *Y*-chromosome followed by crossing and selection of types with particular combinations of chromosomes.

An effort was made in the present study to obtain analyses of 20 complements from each collection. These samples are large enough to reveal some smaller differences between similar complements and, though they are far from perfect, the results obtained have left the writer in a rather critical state of mind regarding statements often encountered in the literature of both plant and animal cytology claiming that complements of different species, races, etc. are the same or identical. Such statements are rarely backed by measurements and accordingly may be misleading in many cases.

The results of chromosome analyses presented in this paper suggest several worth-while studies. First, an attempt to hybridize flies of *H. brassicae* from Guelph with those from some other part of Canada should show whether or not a reproductive isolation barrier exists between these populations and its extent if it exists. Second, a similar study using *H. brassicae* and *H. planipalpus* would be interesting for the same reasons. Third, it would be interesting to test for the presence of an isolation barrier between *H. crucifera* and *H. floralis* since their chromosome complements are quite similar and morphological similarities also exist. Such crosses should provide evidence for or against the writer's view that consistent differences between the complements of two separate populations of the higher Diptera indicate the existence of an isolation barrier between the two populations, though the extent of the barrier cannot be determined through measurement of such differences.




FIGS. 1–12. Somatic chromosome complements of *Hylemya* species at metaphase in brain cells, ×1725. FIG. 1. *H. antiqua* from onion at St. Jean, Quebec. FIG. 2. *H. brassicae* from radish at Belleville, Ontario. FIG. 3. *H. cilicrura* from turnip at Brandon, Manitoba. FIG. 4. *H. cilicrura* from the same source; note large supernumerary (labelled *S*). FIG. 5. *H. cilicrura* from the same source; note three small supernumeraries. FIG. 6. *H. cilicrura* from the same source; note minute supernumerary. FIG. 7. *H. crucifera* from turnip at Brandon, Manitoba. FIG. 8. *H. floralis* from Elgin, Scotland. FIG. 9. *H. fugax*, male, from rotting cabbage at Wye, England; note large *X*-chromosome ($X_1$), small *X*-chromosome ($X_2$), and *Y*-chromosome (*Y*). FIG. 10. *H. fugax*, female, from the same source; note two $X_1$-chromosomes and two $X_2$-chromosomes. Fig. 11. *H. planipalpus* from radish at Brandon, Manitoba. FIG. 12. *H. trichodactyla* ? from turnip at Cloverdale, B.C.

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# STUDIES OF THE MECHANISM OF TRANSMISSION OF POTATO VIRUS Y BY THE GREEN PEACH APHID, *MYZUS PERSICAE* (SULZ.) (HOMOPTERA: APHIDAE)[1]

By R. H. E. Bradley[2]

## Abstract

Wingless adults of *Myzus persicae* (Sulz.) usually ceased to be infective within minutes and always within hours after leaving tobacco infected with potato virus Y, the time being longer when the aphids were kept from feeding than when they fed, and aphids that were kept from feeding remained infective longer at 2° C. than at 35° C. After one to four hours without food, over 80% of *M. persicae* made initial feeding punctures that lasted less than a minute, and about 70% of these aphids transmitted potato virus Y after one such feeding puncture on an infected plant. Though virus was acquired by aphids during feeding punctures as brief as five seconds, those that made feeding punctures lasting 11–60 sec. were the most likely to become infective. The percentage of aphids that transmitted potato virus Y decreased when the stylets were inserted into infected plants for over a minute, and none of the aphids transmitted the virus after the stylets had been inserted over 20 min. The highest percentage of aphids transmitted potato virus Y when they were transferred to test plants immediately after a single brief feeding puncture on an infected plant. The percentage of aphids that transmitted the virus decreased when they spent 10 min. or longer on infected plants; also, the probability of their becoming infective during a single feeding puncture decreased by about one third during the first 10 min. they fed after one to four hours without food. Even when conditions were suitable, about 25% of *M. persicae* failed to transmit potato virus Y. Yet those that failed to transmit the virus in one infection trial transmitted it as readily in a second trial as those that transmitted it in the first.

## Introduction

Many aphid-transmitted viruses are readily acquired by their aphid vectors within minutes or even seconds after the stylets are inserted into infected plants, after which the aphids can immediately infect healthy plants in equally brief periods. Over half of the aphid-transmitted viruses known today are transmitted in this manner, and these cause economic losses in every country, to such crops as: bananas, broccoli, cereals, cucurbits, legumes, oranmental flowers, potatoes, raspberries, strawberries, sugar beets, sugar cane, and tobacco. With perhaps one or two exceptions, it has not been found practical to control the spread of such viruses in the field by the use of insecticides to control the vectors.

At first, this type of transmission by aphids was thought to be a simple mechanical transfer of infective sap on the mouth parts of the vectors (4, 6), but later evidence appears to conflict with this hypothesis and more complex virus–vector relationships have been postulated (11, 12, 13, 14, 15). The evidence for and against these various hypotheses has been discussed elsewhere (1, 10), but, regardless of which one is accepted for the present, there is clearly much to be learned about this type of transmission by aphids. The

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author feels that a better understanding of this type of transmission may lead to better methods of practical control. It was with this in mind that the studies with potato virus Y and the green peach aphid were started. Watson and Roberts (14) showed that potato virus Y is transmitted by aphids in the manner under consideration. As a basis for future studies, the first experiments were designed to learn how long aphids remain infective with potato virus Y, when aphids acquire virus from infected plants, and whether all of the aphids in the population being used were capable of transmitting this virus.

## Materials and General Methods

The strain of potato virus Y (PVY) used throughout the investigation was derived from an infected Green Mountain potato plant supplied by Dr. D. J. MacLeod of the Canada Plant Pathology Laboratory at Fredericton. PVY was transmitted to about 20 young tobacco plants by single aphids that had spent one minute on the original infected potato plant. Since all of the infected tobacco plants developed similar symptoms, one was selected as a future source of PVY. In tobacco, this strain of PVY causes a clearing of the veins in the younger leaves six to eight days after infection; later these leaves become chlorotic with dark green bands along the veins, and growth of the plant is retarded. The symptoms in several varieties of potato are as follows: in Green Mountain there is sometimes slight falling of the lower leaves, but the main symptom is a rugose mosaic on the younger leaves; in Craig's Defiance there is a chlorosis and falling of the lower leaves; in King Edward there is some necrosis of the falling leaves followed by a severe mosaic of the younger leaves; in Majestic there is a necrosis and severe leaf drop streak of the lower leaves together with a mosaic of the younger leaves; and in Katahdin and U. S. D. A. seedling 41956 usually the only symptom is a necrosis that spreads and kills infected plants.

Aphids were obtained from a culture of *Myzus persicae* (Sulz.) reared on virus-free turnips or cabbage at the Entomology Laboratory at Fredericton. Usually wingless adults were used, but occasionally it was necessary to include a few third or fourth instar nymphs. The aphids were collected in cellophane-covered dishes similar to those used by Watson (11), and were always gently handled one at a time with a camel's-hair brush from which all but a few of the hairs had been removed. Unless otherwise stated, all aphids were kept in the collecting dishes from one to four hours without food before they were placed on the source of infection (14).

All transmissions were to and from White Burley tobacco (*Nicotiana tabacum* L.). Plants were raised and maintained in a screened greenhouse, which was fumigated regularly. Though over three thousand plants were used, not one developed symptoms of infection with PVY other than during the period of 5 to 15 days after they were used in an experiment. Furthermore, no accidental infections were found in large numbers of healthy plants kept in the same greenhouse.

The source of infection for aphids was the mature leaves from medium-aged tobacco plants showing well developed symptoms of infection with PVY. These leaves were rubbed gently with a moist cloth or the index finger wet with water to brush down the leaf hairs, which sometimes hinder the aphids in attempting to feed. After a period without food, *M. persicae* usually touches its proboscis to the leaf for a few brief periods before it settles down to continuous feeding (2). The aphids insert their stylets a short distance into the leaf during these brief periods (2), but it is not known whether they imbibe food or not. Therefore, this act by aphids will be called a *feeding puncture* rather than a *feeding period*. *Infection-feeding punctures* are those made on infected plants.

Aphids were tested for infectivity singly or in pairs on young healthy tobacco plants in the three-to five-leaf stage. The aphids were placed on the most mature leaf that measured over one centimeter in length, because experience has shown that they most readily attempt to feed on these leaves. During the test-feeding period, which was over night unless otherwise stated, the plants were covered with glass tumblers to prevent the aphids from wandering away. The plants were fumigated with nicotine fumes to kill the aphids when the tumblers were removed.

## Time that *M. persicae* Remains Infective

In a preliminary experiment to learn how long *M. persicae* remains infective with PVY, aphids were placed on infected plants for about two minutes and then transferred in pairs to a series of three test plants. Each pair of aphids spent one hour on the first test plant, one hour on the second, and 24 hr. on the third. Five pairs of aphids were tested in this manner eight times. The numbers of first, second, and third test plants infected out of 40 were 32, 6, and 0 respectively. Hence, most of the aphids ceased to be infective during the first hour, and none remained so over two hours.

In another experiment, pairs of aphids were transferred to two test plants in succession after two minutes on a source of infection. The time that each pair spent on the first plant was varied from five minutes to 24 hr., and the

TABLE I

INFECTIVITY OF PAIRS OF *M. persicae* FEEDING ON TWO HEALTHY TOBACCO PLANTS IN SUCCESSION

| | Time on first test plant | | | | |
|---|---|---|---|---|---|
| | 5 min. | 15 min. | 30 min. | 1 hr. | 24 hr. |
| First test plants infected out of 30 | 27 | 26 | 25 | 26 | 26 |
| Second test plants infected out of 30 (Test feeding 24 hr.) | 21 | 12 | 2 | 1 | 0 |
| Percentage of infectivity lost on first test plant | 22 | 54 | 92 | 96 | 100 |

time on the second plant was 24 hr. in each case. Five pairs of aphids were tested for each period, and the experiment was repeated six times. The results (Table I) show that over 20% of the aphids ceased to be infective during the first five minutes of the test-feeding period, over 50% were noninfective after 15 min., and over 90% were noninfective after 30 min. Table I also shows that as many infections occurred during the first five minutes of the test feeding period as when the aphids spent up to 24 hr. on the test plants.

Fig. 1 shows the results of an experiment where a comparison was made between the loss of infectivity by aphids allowed to feed and those kept from feeding after leaving the source of infection. After two minutes on an infected plant, the aphids were divided into two equal groups, one group being placed on a healthy plant and the other in a glass dish without food. Five aphids from each group were tested singly for infectivity after periods of 5, 15, 30, and 60 min. This experiment was repeated eight times to make a total of 40 replications of each treatment. There was a rapid loss of infectivity during the first 15 min. by both the aphids kept from feeding and those allowed to feed, followed by a slower loss of infectivity. However, throughout the period, loss of infectivity was more rapid in the aphids that fed than in those kept from feeding.

To learn how long *M. persicae* may remain infective when they do not feed after leaving infected plants, aphids were given two minutes on a source of infection, and then kept in dishes for periods up to 16 hr. before being tested for infectivity. The results of two series of experiments are shown in Table II. In the first series, the aphids were kept in the dishes at room temperatures and tested for infectivity in pairs, while in the second they were kept at 2° C. and at 35° C. and tested singly for infectivity. Though occasional

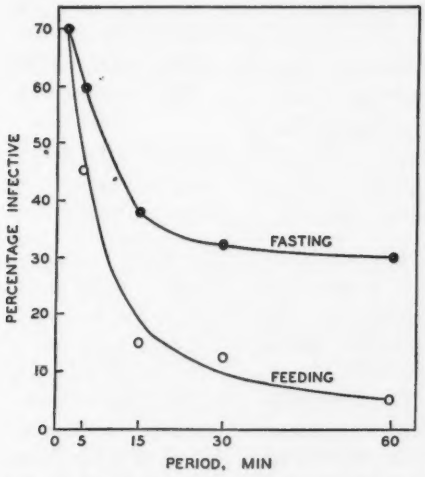


FIG. 1. Percentages (out of 40) of *M. persicae* that transmitted PVY after fasting and after feeding for various periods after removal from source of infection.

TABLE II

NUMBERS OF *M. persicae* OUT OF 30 THAT CAUSED INFECTION WHEN KEPT FROM FEEDING AT DIFFERENT TEMPERATURES FOR VARIOUS PERIODS AFTER LEAVING INFECTED PLANTS

| Temperature aphids kept at | Aphids per test plant | Time kept from feeding after leaving infected plants, hr. | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | ½ | 1 | 2 | 4 | 8 | 16 |
| 20–25° C. | 2 | 23 | 10 | 5 | 2 | 0 | 1 | 0 |
| 2° C. | 1 | 18 | 10 | 5 | 4 | 2 | 1 | 1 |
| 35° C. | 1 | 18 | 2 | 3 | 3 | 1 | 0 | 0 |

aphids remained infective over eight hours, over 60% ceased to be infective during the first hour after leaving infected plants. One aphid caused infection 16 hr. after leaving the source of infection. Loss of infectivity was slower in the aphids kept at 2° C. than in those kept at 35° C.

## Acquisition of Virus

To learn more precisely when PVY is acquired by *M. persicae*, single aphids were tested for infectivity after one infection-feeding puncture. Each aphid was carefully observed with a hand lens while on the infected plant, and a stop watch was used to measure the time that the proboscis remained in contact with the leaf. From 25 to 50 aphids were tested in this manner from July 1951 to June 1952, during which time a total of 1500 aphids were tested. The durations of the infection-feeding punctures and the results of the tests were as follows:

| Duration of infection-feeding puncture, in sec. | 5–10 | 11–20 | 21–30 | 31–40 | 41–60 |
|---|---|---|---|---|---|
| Aphids observed | 95 | 491 | 336 | 169 | 129 |
| Percentage infective | 38.9 | 59.5 | 61.3 | 60.4 | 58.1 |

| Duration of infection-feeding puncture, in min. | 1–5 | 6–10 | 11–20 | 21–60 | Over 60 |
|---|---|---|---|---|---|
| Aphids observed | 185 | 27 | 32 | 25 | 11 |
| Percentage infective | 50.3 | 29.6 | 9.4 | 0 | 0 |

Over 50% of the infection-feeding punctures lasted less than 30 sec., and over 80% terminated in less than a minute. Some aphids became infective during infection-feeding punctures as brief as five seconds. Aphids that made infection-feeding punctures lasting 5–10 sec. were less than 40% infective compared with about 60% for those that made infection-feeding punctures lasting 11–60 sec. The percentage of aphids that transmitted PVY decreased when the infection-feeding puncture lasted over a minute, so that none transmitted the virus when the infection-feeding puncture lasted over 20 min.

In another experiment, single aphids were disturbed with a camel's-hair brush after the proboscis had touched the infected leaf for 5, 10, 15, and 20

sec.; and a fifth group was allowed to complete the infection-feeding puncture undisturbed. Five aphids were tested in each group, and the experiment was repeated seven times. None of the aphids disturbed at five seconds transmitted the virus, but those disturbed at 10, 15, and 20 sec. were 17, 41, and 71% infective respectively. Those disturbed at 20 sec. were as likely to transmit the virus as those that completed the infection-feeding puncture undisturbed (the undisturbed aphids were 69% infective). Only four of the aphids not disturbed made infection-feeding punctures that lasted over 30 sec., which suggests that over 80% of those disturbed would have withdrawn their stylets in less than 30 sec. Therefore, most of the aphids probably acquired PVY during the latter half of the infection-feeding puncture.

Then the probability of transmission after multiple infection-feeding punctures was investigated. Again single aphids were carefully observed with a hand lens while they were on an infected leaf, and tested for infectivity after one, two, or three infection-feeding punctures. The durations of all of the infection-feeding punctures were recorded as in previous experiments. Approximately 10 aphids were tested for each of the three groups on 12 occasions. The total number tested after one, two, and three infection-feeding punctures was 106, 104, and 105 respectively. The following results show that over 80% of the first three infection-feeding punctures lasted less than five minutes, though the second and third infection-feeding punctures tended to last longer than the first:

| Duration of infection-feeding puncture, in min..... | Under 1 | 1–5 | 6–20 | Over 20 |
|---|---|---|---|---|
| First infection-feeding puncture, %.... | 77.6 | 18.9 | 1.3 | 2.2 |
| Second infection-feeding puncture, %.. | 61.5 | 23.9 | 8.3 | 6.3 |
| Third infection-feeding puncture, %... | 54.3 | 28.6 | 11.4 | 5.7 |

The results of multiple infection-feeding punctures showed that once an aphid made a feeding puncture that lasted several minutes, it did not always continue to do so. For example, in 20 cases in which an infection-feeding puncture lasted over five minutes, only five of the feeding punctures that immediately followed lasted over five minutes. The percentages of aphids that transmitted after one, two, and three infection-feeding punctures were 63, 64, and 68 respectively. When only the aphids that made all infection-feeding punctures lasting less than five minutes are considered, the corresponding percentages that transmitted were 67, 69, and 84 respectively. In comparison, those that made one or more infection-feeding punctures lasting over five minutes were less than 30% infective. Aphids that made infection-feeding punctures that lasted over 20 min. followed by others that lasted less than five minutes were over 60% infective. Of 16 aphids that were tested after the final infection-feeding puncture had exceeded 20 min., not one transmitted PVY even though in most cases each aphid had previously made one or two brief infection-feeding punctures.

In a further experiment, the aphids were divided into three equal groups and tested singly for infectivity after various periods on infected and healthy plants. The aphids in the first group were allowed one infection-feeding puncture each, immediately transferred to a second infected plant, and tested for infectivity after 5, 10, or 20 min. Those in the second group were first placed on healthy plants for 5, 10, or 20 min., after which each aphid was given one infection-feeding puncture before it was transferred to a test plant. Those in the third group were transferred to healthy plants immediately after one infection-feeding puncture, and then transferred to test plants after 5, 10, or 20 min. Aphids were also transferred to test plants immediately after a single infection-feeding puncture. This made a total of 10 subgroups, five aphids being tested for each subgroup. The results of 12 replications of this experiment (Table III) show that the greatest number of aphids transmitted PVY when they were transferred to test plants immediately after the initial infection-feeding puncture. When aphids spent 5, 10, or 20 min. on a second source of infection, about as many transmitted PVY after five minutes as after the initial infection-feeding puncture, but the percentage of transmissions after 10 and 20 min. decreased progressively. When an aphid first spent a period on a healthy plant and then made an infection-feeding puncture, the percentage that transmitted PVY decreased from about 75% to about 50% during the first 10 min. and then remained constant up to 20 min. The results for the third group are similar to those in the previous experiments, and show that aphids rapidly lose infectivity when allowed to feed after leaving the source of infection.

TABLE III

NUMBERS OF *M. persicae* OUT OF 60 THAT TRANSMITTED POTATO VIRUS Y AFTER VARIOUS PERIODS ON INFECTED AND HEALTHY PLANTS

| Treatment before aphids were tested for infectivity | Time before aphids were tested for infectivity | | | |
|---|---|---|---|---|
| | One infection-feeding puncture | 5 min. | 10 min. | 20 min. |
| Placed on infected leaf | 47 | 46 | 41 | 26 |
| Placed on healthy leaf and then allowed one infection-feeding puncture | | 40 | 29 | 31 |
| Allowed one infection-feeding puncture and then placed on healthy leaf | | 33 | 29 | 7 |

## Transmissions in Two Successive Infection Trials

Even when conditions were suitable in the previous experiments, usually about 25% of *M. persicae* failed to transmit PVY. To learn whether aphids that failed to transmit virus in one trial would also fail in subsequent trials, individual aphids were carefully passed through the following sequence of events: kept without food 30 min.; given first infection-feeding puncture;

placed on first test plant 30 min.; kept without food 30 min.; given second infection-feeding puncture; placed on second test plant 30 min.; kept without food 30 min.; and placed on third test plant 24 hr. Only one out of 150 aphids tested in this manner infected the third test plant, showing that only rarely were aphids infective after a 30 min. test-feeding period followed by 30 min. without food. During the first two hours of the trial, each aphid made two infection trials. Out of a total of 300 infection trials, there were 244 transmissions, i.e., the probability of transmission during a single trial was 244/300, or 0.83. It was assumed that all of the aphids were capable of transmitting the virus, and the expected frequencies of transmissions during two trials were calculated on the basis that $p = 0.83$. The expected and observed frequencies of transmissions were as follows:

| Test plants infected in..... | Both trials | First trial | Second trial | Neither trial |
|---|---|---|---|---|
| Transmissions observed..... | 97 | 28 | 22 | 3 |
| Transmissions expected ($p = 0.83$).... | 98.4 | 23.1 | 23.1 | 5.4 |

Aphids that failed to transmit in the first trial transmitted as readily in the second trial as those that transmitted in the first. Also, the expected and observed frequencies agreed very closely with one another. These results indicate that all of the aphids in the culture being used were capable of transmitting PVY.

## Discussion

The results of experiments on the time that *M. persicae* remains infective with PVY are very similar to those obtained with henbane mosaic (12), tobacco etch (7), and sugar beet mosaic (13) viruses. With each of these, *M. persicae* usually ceases to be infective within minutes and always within hours after leaving infected plants, the time being longer if the aphids do not feed after leaving the source of infection. Also, aphids that do not feed after leaving the source of infection remained infective with PVY longer at 2° than at 35° C., a result similar to that obtained with tobacco etch virus (7). Hence, these results further confirm that the viruses named above are transmitted in essentially the same way by their common vector, *M. persicae*.

The main point to be discussed is the bearing of the results presented here on the mechanism of transmission of viruses like PVY by their aphid vectors. Earlier workers believed that such viruses were carried as contaminants on the mouth parts of aphids, largely because they were transmitted by their vectors within a few minutes and infectivity was soon lost. Watson (11, 12, 13) and later Watson and Roberts (14, 15), working with henbane mosaic and other viruses, demonstrated that this type of transmission by aphids was more complex than had been thought previously. They showed that vector efficiency was increased greatly if the aphids were first kept without food for a period and then only allowed a few minutes at most on infected plants. Watson and Roberts (14) felt that it was not necessary to postulate a different

mechanism of transmission for these viruses from that of other insect-transmitted viruses. Instead, they postulated that viruses that do not persist in their vectors are inactivated by substances produced by the vectors, and that these substances are not produced or produced in much smaller quantities when the aphids are not feeding. In presenting this hypothesis, Watson and Roberts (14, 15) strongly opposed the belief that such transmission was of a mechanical nature. These two hypotheses are not, however, mutually exclusive for the results with PVY indicate that each may be right in some respects.

It was during brief feeding punctures into infected plants that *M. persicae* became infective with PVY. Transmissions rarely occurred after infection-feeding punctures lasting 10 min. or longer, and none of the aphids that inserted their stylets for over 20 min. transmitted PVY. Furthermore, after several hours on infected plants, aphids that had their stylets inserted more than 25 $\mu$ did not transmit the virus (3). During brief feeding punctures, such as those when aphids become infective with PVY, the stylets are only inserted into the epidermis, and little or no saliva is ejected (2). It is clear then that *M. persicae* acquires from the epidermis PVY that can later be used to infect plants. At present, there is no proof that aphids ever become infective with PVY after their stylets have passed through the epidermis of an infected plant. Viruses like PVY may exist in different states in a plant, so that only virus in the epidermis is in a state that can be transmitted by aphids, but other explanations of these results are also possible.

It is not known how soon an aphid imbibes food after inserting the stylets into a plant. Aphids that have been kept without food might be expected to feed as soon as possible, but, if so, they would hardly be expected to withdraw their stylets in less than a minute as is usually the case. The early termination of the first few feeding punctures suggests that feeding at such times is not normal. Normally when *M. persicae* feeds, the stylets are inserted as far as the phloem and are surrounded by a tubular salivary sheath (8, 9). In comparison, little or no saliva is ejected during the brief feeding punctures when aphids become infective with PVY. This suggests that the salivary sheath may, in some way, prevent aphids from becoming infective with PVY. Though the chemical and physical properties as well as the function of the salivary sheath are poorly understood, probably the materials ingested by aphids pass through the sheath. This in itself offers opportunities for the sheath to prevent the aphids from becoming infective. For example, the pores of the sheath may be too small to allow the passage of viruses like PVY, or such viruses may be adsorbed on the sheath. Or, if inactivating substances affect this type of transmission in the manner postulated by Watson and Roberts (14, 15), then these may be present in the saliva. Still another manner in which the salivary sheath may prevent aphids from becoming infective has been postulated by Bradley (2). Regardless of which of these hypotheses most closely depicts this type of transmission, such transmission may still be of a mechanical nature in that virus is carried *in* or *on* the mouth parts. Nor does a belief in a mechanical type of transmission necessarily imply that the

stylets merely act like needles on which virus is carried as a contaminant. The specificity between some viruses transmitted in this manner and their aphid vectors clearly indicates that the complexity of this type of transmission is not yet fully understood.

There are similarities between this type of transmission by aphids and the transmission of myxoma virus of rabbits by the mosquito *Aedes aegypti* (L.). Although active virus could be demonstrated in the bodies of *A. aegypti* that had ingested blood from infected rabbits, the mosquitoes failed to transmit the virus unless they had first inserted their stylets into a surface skin lesion on the infected rabbit (5). Fenner, Day, and Woodroofe concluded from their results that mosquitoes transmitted myxoma virus only after the stylets became contaminated with virus as they probed into surface skin lesions. Hence, aphid vectors of PVY and mosquito vectors of myxoma virus both become infective when the stylets are inserted superficially into infected hosts.

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# THE EFFECT OF INSECTICIDES ON THE ACTION POTENTIALS OF INSECT NERVE[1]

By D. I. V. Lalonde and A. W. A. Brown


## Abstract

Solutions of 17 insecticides in corn oil were applied to the leg of *Periplaneta americana* (L), and their effect on the sensory impulses of the crural nerve was determined by means of a preamplifier and cathode-ray oscillograph. DDT, methoxychlor, and DDD induced well-marked trains of repetitive discharge. Aldrin and dieldrin evoked low-voltage trains of repetitive discharge after a latent period of two to four hours. Technical chlordane, heptachlor, $\alpha$-chlordan, $\beta$-chlordan, and toxaphene all showed considerable latent periods before stimulating the action potentials. Lindane rapidly induced the appearance of tiny sets of repeat spikes. The pyrethrins increased the frequency of impulses, while the dinitro compounds increased both the voltage and frequency. Schradan showed no effect, TEPP a transient stimulation, while parathion induced trains of repetitive discharge after a latent period of three hours.


## Introduction

The appearance of the cathode-ray oscillograph has furnished a convenient method whereby the action of insecticides on nerve transmission may be observed visually, and permanently recorded by photography. Using this method, Welsh and Gordon (9) and Roeder and Weiant (7, 8) have concluded that the characteristic effect of DDT on the nervous system of *Periplaneta americana* is the replacement of a single impulse by a train of multiple impulses recurring at a frequency of 200–500 per second. In the present work, this type of investigation is extended to cover 17 organic insecticides, including DDT; but instead of perfusing the nerve, the insecticide was applied by contact to the surface of the leg, and the oscillograph record was taken from the crural nerve isolated from its connection with the central nervous system so that only sensory impulses were involved.

## Material

Adult males of the American cockroach, *Periplaneta americana* (L.), were used as the test insects and were taken between one week and two months after the final molt. Full information on the 17 insecticides employed are shown in Table I. These were dissolved in corn oil (Mazola, Canada Starch Co.) such that 100 cc. of the oil contained 1 gm. of the active compound.

## Methods

Nerve potentials were taken from the crural nerve of the metathoracic leg and amplified on a Grass P4 preamplifier before being displayed on a du Mont 304H oscillograph screen. Photographs were obtained with a Leica 35 mm. camera fitted with a portrait lens.

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TABLE I

SOURCE AND FULL DESIGNATION OF INSECTICIDAL MATERIALS EMPLOYED

The first 12 materials listed were recrystallized in the laboratory before use

| Insecticide | Fuller designation | Purity | Source |
|---|---|---|---|
| DDD | *p,p'*-Dichlorodiphenyldichloroethane | Technical | Rohm & Haas Co. |
| DDT | *p,p'*-Dichlorodiphenyltrichloroethane | Purified | Experimental Sta., Suffield |
| Methoxychlor | *p,p'*-Dimethoxydiphenyltrichloroethane | Technical | Du Pont Experimental Sta. |
| Lindane | gamma-Hexachlorocyclohexane | Purified | Experimental Sta., Suffield |
| Toxaphene | Chlorinated Camphene | Technical | Hercules Powder Co. |
| α-Chlordan | *cis*-Octachlorohexahydromethanoindene | Purified | Julius Hyman & Co. |
| β-Chlordan | *trans*-Octachlorohexahydromethanoindene | Purified | Julius Hyman & Co |
| Heptachlor | Heptachlorotetrahydromethanoindene | Purified | Julius Hyman & Co. |
| Aldrin | Hexachlorodiendomethanohexahydronaphthalene | Purified | Julius Hyman & Co. |
| Dieldrin | Epoxy derivative of Aldrin | Purified | Julius Hyman & Co. |
| DNOC | 3,5-Dinitro-ortho-cresol | Technical | Standard Agric. Chem. |
| DNCHP | 3,4-Dinitro-6-cyclohexylphenol | Purified | University of Toronto |
| TEPP | Tetraethyl pyrophosphate | 40% in phosphates | Monsanto Chem. Co. |
| Parathion | Diethyl-*p*-nitrophenyl thiophosphate | 99.8%* | American Cyanamid Co. |
| Schradan | Octamethylpyrophosphoramide | 99% | Pest Control Ltd. |
| Pyrethrins | Pyrefume Super 100 | 12% in kerosene | S. B. Penick & Co. |
| Tech. Chlordane | Mixture of chlordane compounds | Refined | Julius Hyman & Co. |

* *Not more than two months from time of manufacture.*

The insects were anesthetized with carbon dioxide, decapitated, and pinned ventrum uppermost on a balsa block. The crural nerve was exposed by removing the integument, fat-body, and tracheae overlying the metathoracic ganglion. It was moistened with a drop of insect saline, containing chlorides of sodium, potassium, and calcium (6). The ganglion and its surrounding connections were crushed with forceps in order to prevent extraneous potentials and muscular activity from appearing on the screen. A pair of silver electrodes (4), whose fine tapered tips were bent into hooks, were brought up under the crural nerve by means of a micromanipulator, such that one was 2 mm. from the crushed base of the nerve and 2 mm. from the other electrode; these electrodes therefore recorded only the potentials emanating from the metathoracic leg.

The 1% insecticide solution was painted on the leg from the tarsus to the trochanter; since the amount thus applied was 3 cu. mm., the dosage was 30 $\mu$gm. The preparation was then observed continuously, being lightly moistened with saline every minute. The observation period was four hours, being extended to nine hours for insecticides known to have a long reaction time (2).

## Results

The effects of the various insecticides, applied to the metathoracic leg, upon the action potentials of the crural nerve, are shown pictorially in Figs. 1 to 3. Each photograph represents a characteristic oscillograph picture based on continuous recording of three to six preparations treated by the insecticide in question.

The action potentials appear as vertical oscillations of the horizontal sweep of the cathode-ray across the screen, the thin lines so produced being called "spikes". Since bipolar recording was employed, each pulse as it travelled along the nerve produced a spike above the base line followed by a similar one below the line. The amplitude of the impulse may be measured in microvolts ($\mu$v.) as the distance the spikes attain either above or below the base line by comparison with traces of calibration impulses of known voltage supplied by a standard source. The time scale is also shown, calibrated from the standard power supply at 60 cycles per second.

At the high levels of amplification used, the most careful screening could not entirely eliminate induction from domestic power sources and automobile ignition systems. This noise caused a slight oscillation of the horizontal trace, which is shown herewith for the lowest and highest amplifications employed (Figs. 1*a* and 1*b*).

### *Control Preparations*

The oscillograph trace from an untreated crural nerve preparation moistened with saline is shown in Fig. 1*c*. It will be seen that spikes of potential occur at irregular intervals, their frequencies ranging from 10 to 50 per sec.; their height seldom exceeds 50 $\mu$v. When the tarsus was treated with 3 cu. mm.

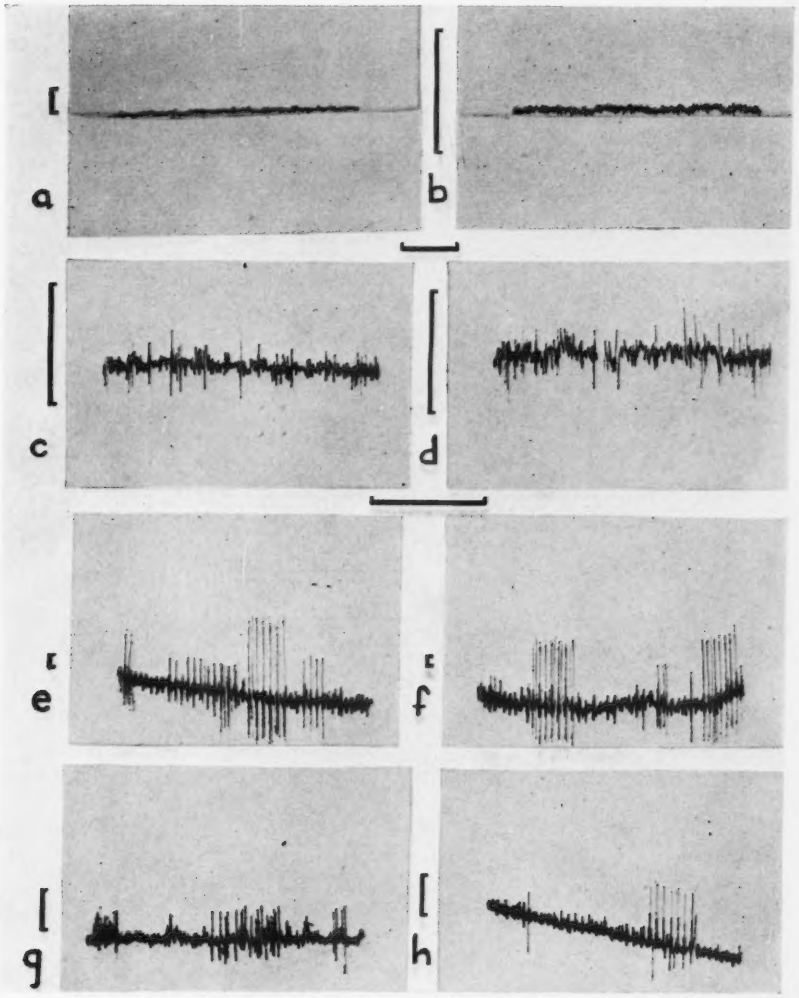


FIG. 1. Action potentials of nerve preparations treated with DDT and analogues, and of control preparations. *a*. Noise level (amplifier 1.5 × 6). *b*. Noise level (amplifier 1.5 × 9). *c*. Untreated. *d*. Corn oil. *e*. DDT (leg free). *f*. DDT (leg contracted). *g*. DDD (leg contracted). *h*. Methoxychlor (leg free). Vertical scales 100 μv. Horizontal scales 0.1 sec.

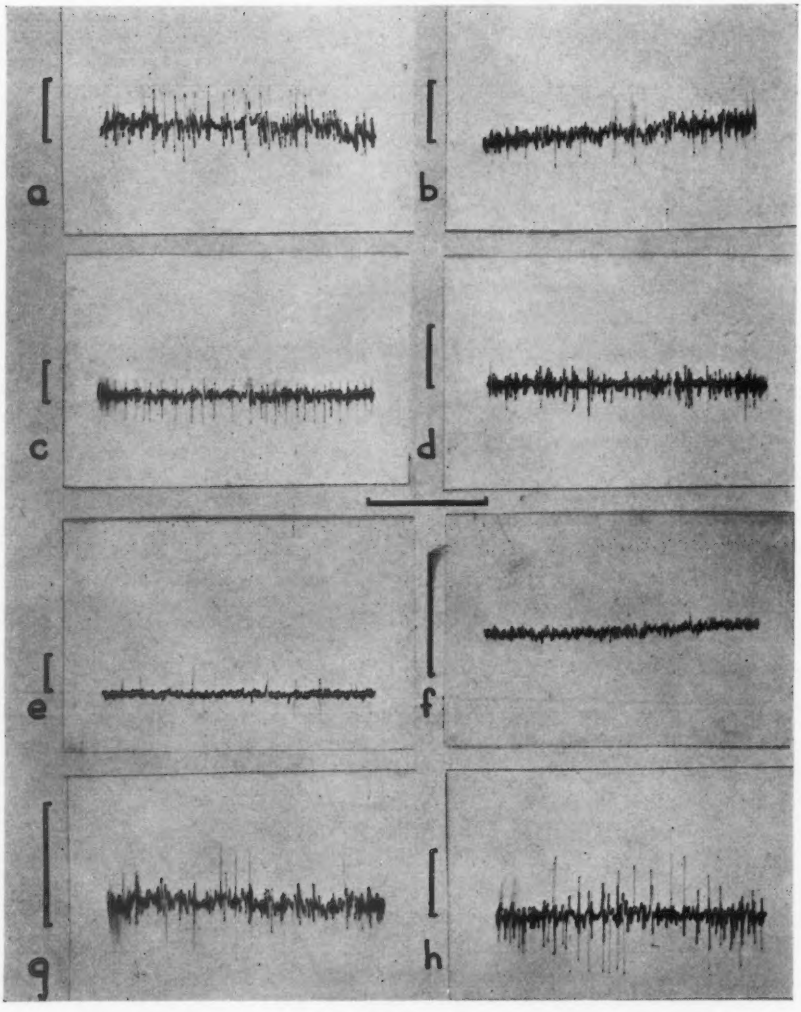


FIG. 2. Action potentials of nerve preparations treated with chlorinated terpenes and lindane. *a*. Aldrin. *b*. Dieldrin. *c*. Toxaphene. *d*. Lindane. *e*. α-Chlordan. *f*. β-Chlordan. *g*. Heptachlor. *h*. Technical Chlordane Vertical scales 100 μv. Horizontal scale 0.1 sec.

of corn oil containing no insecticide, the ensuing oscillograph trace was not found to be significantly different, the spike voltages and frequencies continuing at the normal level (Fig. 1*d*).

*Chlorinated Hydrocarbons*

The effect of DDT and its two analogues on the crural nerve was to produce trains of repetitive discharge one to two hours after being applied to the leg, which continued for at least three hours (Fig. 1*e*). Although the trains appeared spontaneously, they were more fully developed if the leg was contracted (Fig. 1*f*). With DDT, each train was of 0.1–0.2 sec. duration, and consisted of a series of spikes 250–700 $\mu$v. in height repeating at the rate of 200–450 per sec.; the trains followed close upon each other at intervals of approximately 0.1 sec. With methoxychlor the picture was similar, except that the level of the spike voltages was only 120 $\mu$v. (Fig. 1*h*). With DDD, the trains appeared sooner (after one hour) and were shorter in duration, their voltage level averaged 90 $\mu$v. and they succeeded each other at intervals of approximately three seconds (Fig. 1*g*).

The effect of lindane, which is not an analogue of DDT, appeared one hour after treatment but it did not take the form of trains of repetitive discharge. Instead there appeared at short intervals not exceeding 0.5 sec., tiny sets of two to four repetitive spikes 50–100 $\mu$v. in height (Fig. 2*d*).

*Chlorinated Terpenes*

All of these compounds were characterized by a latent period of several hours, ranging from two hours with dieldrin to eight hours with $\alpha$-chlordan, which preceded the appearance of repetitive discharge of some kind.

With dieldrin (Fig. 2*b*), the repetitive discharge appeared in short trains (not exceeding 0.2 sec.) of low-voltage spikes (50 $\mu$v.). On first appearing two hours after treatment, these trains showed a frequency of 240 per second but increased in the next hour to 480 per second, returning to the original frequency in the fourth hour. With aldrin, similar trains appeared after a latent period of four hours (Fig. 2*a*); the spikes were again 50 $\mu$v. in height and the frequency was 180 increasing to 240 per second.

When toxaphene was applied to the leg, repetitive discharge appeared after a three-hour latent period (Fig. 2*c*). This took the form of 100-$\mu$v. spikes, repeating at the rate of 180 per second continuously for approximately 30 min., after which all electrical activity ceased in the nerve.

Technical chlordane, a mixture of insecticidal compounds, induced trains of low-frequency spikes after a five-hour latent period (Fig. 2*h*). The duration of the trains was 0.2–0.3 sec., consisting of 60-$\mu$v. spikes repeating at the rate of 240 per second. Heptachlor, one highly insecticidal constituent, induced groups of repetitive (180 per second) spikes after a latent period of three hours (Fig. 2*g*). The constituent called $\beta$-chlordan markedly depressed the level of potentials during a three-hour latent period, after which certain preparations showed high-voltage spikes, or high-voltage trains on stimulation

(Fig. 2*f*). Its less insecticidal isomer, α-chlordan, depressed the action potentials much more gradually during an eight-hour latent period, at the end of which time approximately half the preparations survived to show small groups of high-voltage spikes for the succeeding three hours (Fig. 2*e*).

*Dinitro Compounds*

Almost immediately after it was applied, the fast-acting insecticide DNOC stimulated the spontaneous discharge of the nerve (Fig. 3*a*). The discharge steadily increased to a point where it induced contraction of the leg, which always occurred within 15 min. from the time the insecticide was applied. At the time of contraction, nonrepetitive spikes of discharge reaching 75–100 μv. in height were following each other at the extremely high frequency of 420 per second (Fig. 3*b*). The spikes then showed a steady decline in frequency and voltage, to disappear entirely 45 min. after the treatment with DNOC.

With DNCHP, the action potentials of the crural nerve similarly increased (Fig. 3*c*) until they induced flexion of the leg 90 min. after application. By this time the spikes were 50–100 μv. in height and followed each other at a frequency approaching 600 spikes per second. This condition persisted for the two to three hours of observation after the leg had contracted.

*Organic Phosphates*

The anticholinesterase TEPP proved to have a transient effect on the electrical activity of the crural nerve. Within one to two hours of application, groups of repetitive spikes appeared, often attaining the frequency of 360 per second (Fig. 3*g*). This response persisted for about 20 min., after which the preparation returned apparently to normal.

Parathion did not affect the nerve preparation for about two hours after application. Then 50–75-μv. spikes appeared which repeated at a frequency of 60–100 per second (Fig. 3*e*). After three hours, these were superseded by trains, of 0.25 sec. duration, of spikes with the uniform voltage of 150 μv. and repeating at the rate of 180–360 per second (Fig. 3*f*).

When the preparation was treated with schradan, no abnormalties in either voltage of frequency of the action potentials could be detected during the ensuing four hours (Fig. 3*h*).

*Botanical Compounds*

When a mixture of pyrethrins was applied to the leg, the action potentials of the crural nerve were stimulated one hour later. The spikes appeared at shorter and shorter intervals, although the voltage remained the same. Three hours after treatment, these spikes often recurred in trains at a frequency of 360 per second. When the leg was forcibly contracted, the voltage of the spikes increased to 110 μv. and the trains became continuous volleys.

## Discussion

Where these results meet on the common ground represented by DDT, they are in substantial agreement with those of previous workers, in that

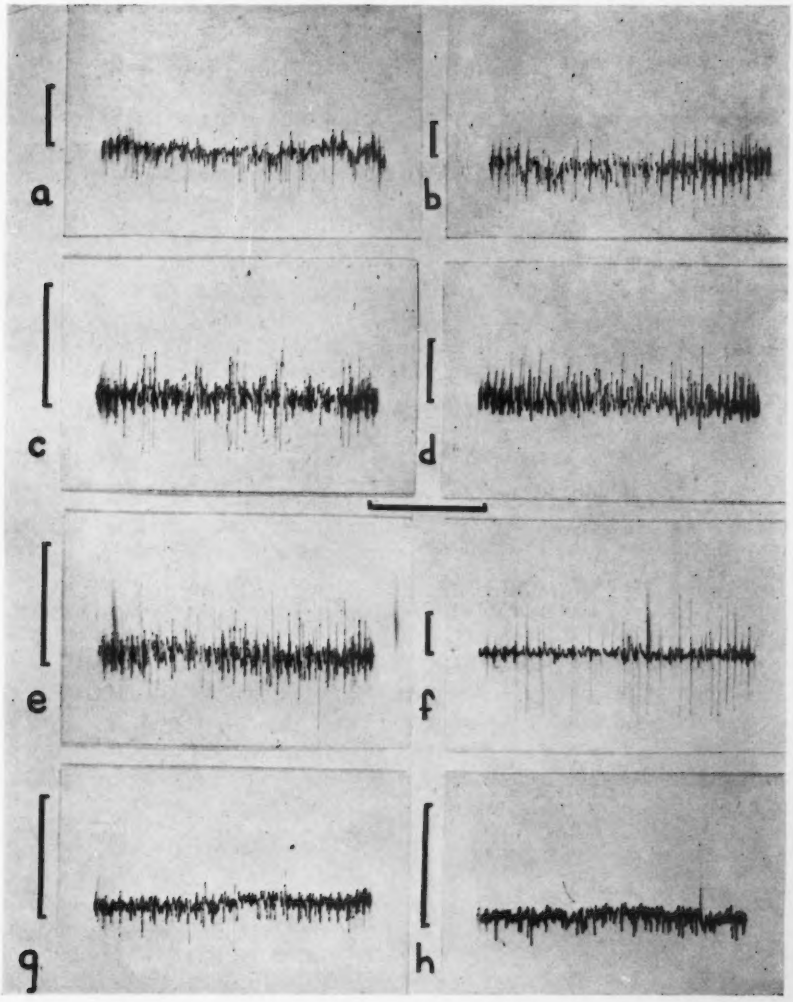


FIG. 3. Action potentials of nerve preparations treated with dinitro compounds, pyrethrins, and organic phosphates. *a*. DNOC (before contraction). *b*. DNOC (after contraction). *c*. DNCHP (before contraction). *d*. Pyrethrins. *e*. Parathion (early stage). *f*. Parathion (late stage). *g*. TEPP. *h*. Schradan. Vertical scales 100 $\mu$v. Horizontal scale 0.1 sec.

similar trains of repetitive discharge could be obtained. The frequency of 200–500 spikes per second observed for the repetitive discharge induced by DDT or methoxychlor agrees with the figures cited by Welsh and Gordon (9). The latent period of 60–120 min. between the application of DDT and the appearance of trains, however, is rather longer than that of 3–35 min. observed by Roeder and Weiant (7,8) in perfusion experiments. DDD and methoxychlor were found to resemble DDT in causing the characteristic trains of repetitive discharge, thus demonstrating that these three chemical analogues bear generic similarities in their action upon nerve. On the other hand, lindane, a chlorinated hydrocarbon of much simpler structure, exerted a quite different effect. No trains of multiple impulses appear, but instead very short bursts of discharge follow rapidly on each other.

These experiments have demonstrated that aldrin and dieldrin also induce trains of repetitive discharge, although of lower voltage and frequency than with DDT. The latent periods before these chlorinated terpenes exerted an effect were longer than with the DDT group, being four hours with aldrin and two hours with dieldrin. This is in remarkable agreement with the latent periods of 250 min. and 100 min., respectively, reported by Harvey and Brown (2) for the latent period preceding the stimulation of respiration of *Blattella germanica* by these compounds.

The latent period before an effect on nervous transmission is exerted also characterizes the other chlorinated terpenes, such as the chlordane compounds. In these experiments, it was found that technical chlordane had no effect on action potentials for approximately five hours, but that trains of high-frequency spikes then appeared, which persisted for several hours. A similar latent period of 300 min. had been observed to precede the stimulatory effect of the chlordane on the respiratory rate of *Blattella germanica* (2). This long delay before this insecticide affected the nerve was possibly responsible for the conclusion of Roeder (5) that technical chlordane did not have an effect on synaptic transmission in *Periplaneta americana*. When the leg was contracted after five hours, the nerve showed an exaggerated response in the form of 100-$\mu$v. spikes, which may be taken to confirm the general experience of entomologists that chlordane-poisoned insects show a hyperexcitability to stimuli.*

The constituent insecticidal compounds in technical chlordane, namely heptachlor, $\beta$-chlordan, and $\alpha$-chlordan, all required a considerable period of time to elapse before they took effect, the latent periods being three, three, and eight hours respectively. Similar latent periods of 150, 250, and 400 min., respectively, had been observed by Harvey and Brown (2) to precede the effect of these compounds in the oxygen consumption of *Blattella*. After the latent period, high-frequency spikes appeared regularly and distinctly with the quicker-acting heptachlor; with the slower-acting chlordane isomers, they appeared only in a proportion of the insects treated with $\alpha$-chlordan and only

* *This property, also shown by mice poisoned with this material, is evidently due to an impurity present in Julius Hyman's technical chlordane but not in that of the Velsicol Corporation.*

very occasionally with β-chlordan, as if they exerted their depressing effect by significantly heightening the threshold of sensitivity of the nerve. The effect of toxaphene was also preceded by a latent period, but it differed by being a continuous volley of spikes.

The dinitro compounds each showed the same effect of increasing the voltage and frequency of impulses and thus inducing flexion of the leg. These findings underline the known rapid action of these insecticides, especially DNOC, and show that their mode of action involves nervous tissue. The pyrethrins, on the other hand, increased only the frequency of impulses. The oscillograph record obtained on contraction of the leg of a treated preparation showed clearly the volleys of high-voltage spikes first observed with pyrethrins by Loewenstein (3).

In the case of the organic phosphates, it would seem a priori unlikely to find that they would exert an effect on the nerve in the absence of a synapse. Indeed, schradan apparently had no effect at all. Whether this is related to the lack of cholinesterase properties in schradan (1) cannot be determined, since it is not known whether the tissues of the cockroach leg convert the insecticide to an anticholinesterase, as rat liver does. The effect of the strong anticholinesterase TEPP was only a transitory stimulation, which arouses the suspicion that it was due to the acid products liberated by hydrolysis of this insecticide in the leg tissue. The fact remains, however, that the sample of parathion employed, of high purity and sufficiently fresh to show little isomerization, did induce a repetitive discharge of trainlike character more closely resembling the DDT effect than the effect of any other insecticide tested; but the latent period of three hours may indicate that molecular changes had taken place in the tissues.

The finding that every insecticide but one induced some effect does not imply that the nerve itself was involved. The spontaneous discharge, on which the effect of the insecticide is investigated, must have some point of origin such as campaniform sensilla on the trochanters which carry the endings of the sensory fibers. It is therefore possible that some of the insecticides may exert their effect on the sensory cells, while others affect the nerve itself; by the method employed it is impossible to distinguish between these two modes of action.

## Conclusions

The effect of methoxychlor and of DDD on the action potentials of the crural nerve of the American cockroach is to produce trains of repetitive discharge, which, however, are not so well developed as when DDT is used. Aldrin and dieldrin induce trains of low-voltage repetitive discharge after latent periods of two to four hours. The chlordane compounds, including heptachlor, α-chlordan, and β-chlordan, cause the appearance of repeat spikes after latent periods of three to eight hours. Toxaphene induces continuous low-frequency repetitive discharge after a latent period of three hours. Lindane evokes tiny sets of two to four repeat spikes. The dinitro compounds DNOC

and DNCHP increase the sensory potentials in voltage and frequency so as to induce contraction of the leg in 15 and 90 min. respectively. Schradan is without effect, TEPP causes a transient stage of repeat spikes, while parathion evokes definite trains of repetitive discharge within three hours. The pyrethrins increase the frequency of spikes until trains of repetitive discharge appear. It may therefore be concluded that all the organic insecticides tested exert an effect on insect nerve, with the exception of schradan.

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# A NUCLEAR AND A CYTOPLASMIC POLYHEDRAL VIRUS DISEASE OF THE SPRUCE BUDWORM[1]

By F. T. Bird and Mary M. Whalen[2]

## Abstract

The spruce budworm, *Choristoneura fumiferana* (Clem.), is susceptible to two polyhedral virus diseases. The first, previously described, is characterized by the formation of polyhedra within the nuclei of tracheal matrix, fat, hypodermal, and blood cells. The polyhedra contain mostly rod-shaped particles 260 × 28 millimicrons. The second is a new type and is characterized by the formation of polyhedra in the cytoplasm of mid-gut cells. The polyhedra contain only spherical particles from 28 to 80 mμ in diameter. Both diseases occurred in the same insect but it was possible to separate the cytoplasmic disease from the nuclear disease by extracting groups of the cytoplasmic polyhedra from the mid-gut with a micromanipulator before the nuclear disease developed.

## Introduction

A polyhedral virus disease of the spruce budworm, *Choristoneura fumiferana* (Clem.), was first reported by Graham (10) who observed that "the true polyhedra most clearly originate in the digestive cells of the mid-gut." Bergold (3, 4) studied purified polyhedra obtained from virus-killed spruce budworm larvae and demonstrated that they contained rod-shaped virus particles 260 × 28 millimicrons, similar to those isolated from polyhedra of other Lepidoptera (1, 2). A histological investigation by Bird (6) showed that polyhedra were formed in the nuclei of tracheal matrix, fat, hypodermal, and blood cells and that granules similar to polyhedra were formed in the cytoplasm of mid-gut cells. Previously, Ishimori (12) reported that, in addition to polyhedra found in the nuclei of other tissues, polyhedra were formed in the cytoplasm of the mid-gut cells of the silkworm, *Bombyx mori* (L.). Lotmar (14) also reported polyhedra formation in the cytoplasm of mid-gut cells of the clothes moth, *Tineola bisselliella* (Hum.), as well as in the nuclei of other cells. Recently, Xeros (18) and Smith and Xeros (17) have reported cytoplasmic polyhedra in the guts of several Lepidopterous larvae.

Studies were continued on the disease of the spruce budworm to determine whether the cytoplasmic polyhedra are associated with an infectious agent, the nature of this agent, and the relation between the cytoplasmic and nuclear diseases.

## Methods

Larvae infected with the original inoculum, obtained from virus-killed spruce budworm larvae, always produced both types of disease: the cytoplasmic in two to three days, and the nuclear in about seven days.

To separate the agent associated with the formation of cytoplasmic polyhedra from that associated with the formation of nuclear polyhedra,

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colonies* of cytoplasmic polyhedra were collected from the mid-gut epithelia of spruce budworm larvae by means of a small pipette attached to a micromanipulator. This was done two to three days after the larvae had been fed virus and before the development of nuclear polyhedra. The colonies were washed several times in sterile distilled water and fed to apparently healthy larvae. The cytoplasmic polyhedra were passaged in this way several times in an attempt to obtain a suspension which would produce only the cytoplasmic disease.

To study the cytoplasmic polyhedra, colonies of these polyhedra were removed from the mid-gut epithelia in the manner described above. Each colony was washed several times in sterile distilled water, placed on an electron microscope screen, and then punctured to allow the contents to spread over the screen. Large numbers of colonies of cytoplasmic polyhedra were collected, the polyhedra were purified, and the particles which they contained were liberated by dissolution of the polyhedra in weak alkali (0.04 *M* sodium carbonate + 0.05 *M* sodium chloride) using a technique similar to that of Bergold (4).

Histological studies were made of the two types of infection. Some larvae were fed polyhedral suspensions which produced both the cytoplasmic and nuclear polyhedra within susceptible cells. Others were fed polyhedral suspensions which produced polyhedra only in the cytoplasm of mid-gut cells. Tissues were fixed in Bouin's fluid and stained with Heidenhain's iron haematoxylin.

In one series of experiments, the infection process was studied by feeding larvae cytoplasmic polyhedra, and, at 24 hr. intervals, removing the guts of the larvae either for examination with the dark-field microscope or for sectioning.

## Histology of the Nuclear Disease and Description of the Virus Particles

The nuclear infection of spruce budworm larvae is typical of polyhedral virus infections described for many Lepidoptera (8,9,11,13,15). Polyhedra are formed within the nuclei of the tracheal matrix, hypodermal, fat, and blood cells. The first symptoms of infection, evident from stained sections, are a swelling of the nucleus and coagulation of the chromatic material of the nucleus. Polyhedra appear as small granules both in the achromatic and chromatic materials, increase in number and size, and form a ring around a central coagulated mass of nuclear materials. The mature polyhedra, which vary in shape but are frequently triangular, average about 2 $\mu$ in diameter. Bergold (4) has shown that they contain mostly rod-shaped particles about 260 $\times$ 28 m$\mu$, and also spherical particles which develop into rods (3). Fig. 1 is a section through the hypodermis of a spruce budworm larva showing nuclei in an early stage of infection. Fig. 2 shows a nucleus of a tracheal matrix cell containing mature polyhedra.

* *"Colony" is used to describe a group of polyhedra of the same size enclosed within a membrane. Biological relationship of the polyhedra is not yet established.*

## Histology of the Cytoplasmic Disease and Description of the Virus Particles

Polyhedra occur in the cytoplasm of the digestive cells of the mid-gut epithelium of spruce budworm larvae as colonies of uniformly dispersed, crystal-like bodies, enclosed by a membrane. The polyhedra in each colony increase in number and in size, and frequently one cell contains more than one colony. The polyhedra average about 0.5 $\mu$ in diameter but occasionally a colony contains polyhedra 3 $\mu$ or more in diameter. Their staining properties with iron haematoxylin, following fixation in Bouin's fluid, are similar to those of the nuclear polyhedra except that the mature cytoplasmic polyhedra are more easily stained. Preliminary studies by G. H. Bergold of this laboratory have shown that like the nuclear polyhedra, the cytoplasmic polyhedra are protein in nature and contain desoxyribonucleic acid. They are chiefly cuboidal and the triangular forms frequently occurring among nuclear polyhedra have never been found among cytoplasmic polyhedra. Fig. 3 is a section through the mid-gut of a spruce budworm larva in an advanced stage of infection. Fig. 4 is a section of one mid-gut cell with cytoplasmic polyhedra surrounding the nucleus of the cell.

Each colony contains, in addition to the polyhedra, a large number of very small spherical particles (Fig. 5). Similar particles, varying in size from 28 to 80 m$\mu$ in diameter (Fig. 6), were isolated from purified cytoplasmic polyhedra when the latter were dissolved in weak alkali. Rod-shaped particles, characteristic of the nuclear disease, have never been isolated from polyhedra of the cytoplasmic disease.

## Experimentation and Occurrence of the Disease in Nature

By removing colonies of cytoplasmic polyhedra from the mid-gut epithelia in the manner described previously, and passaging them three times in the host larvae, a suspension of cytoplasmic polyhedra was obtained which, when fed to spruce budworm larvae, produced only the cytoplasmic disease (Table I).** The results of this experiment were based on the ability to distinguish, under the dark-field microscope, the two types of diseases by the differences in the size and shape of the polyhedra. From the polyhedra collected, only the small spherical particles typical of the cytoplasmic disease were isolated, indicating that the diagnoses were correct.

Dark-field microscope studies of the guts of 190 infected larvae and 147 control larvae showed colonies in 99% of the infected larvae 72 hr. after infection, and no colonies in the guts of the control larvae reared up to 12 days. Similarly, the study of sections showed that colonies of polyhedra were formed in the cytoplasm of mid-gut cells of 28 larvae 72 hr. after infection (some were slightly infected after 48 hr.), and no colonies in the guts of 29 control larvae reared up to 21 days.

*** The larvae used in this experiment were not free from disease and small percentages of the control larvae died from the cytoplasmic disease. Considerable numbers died from diseases other than the cytoplasmic and nuclear diseases.*

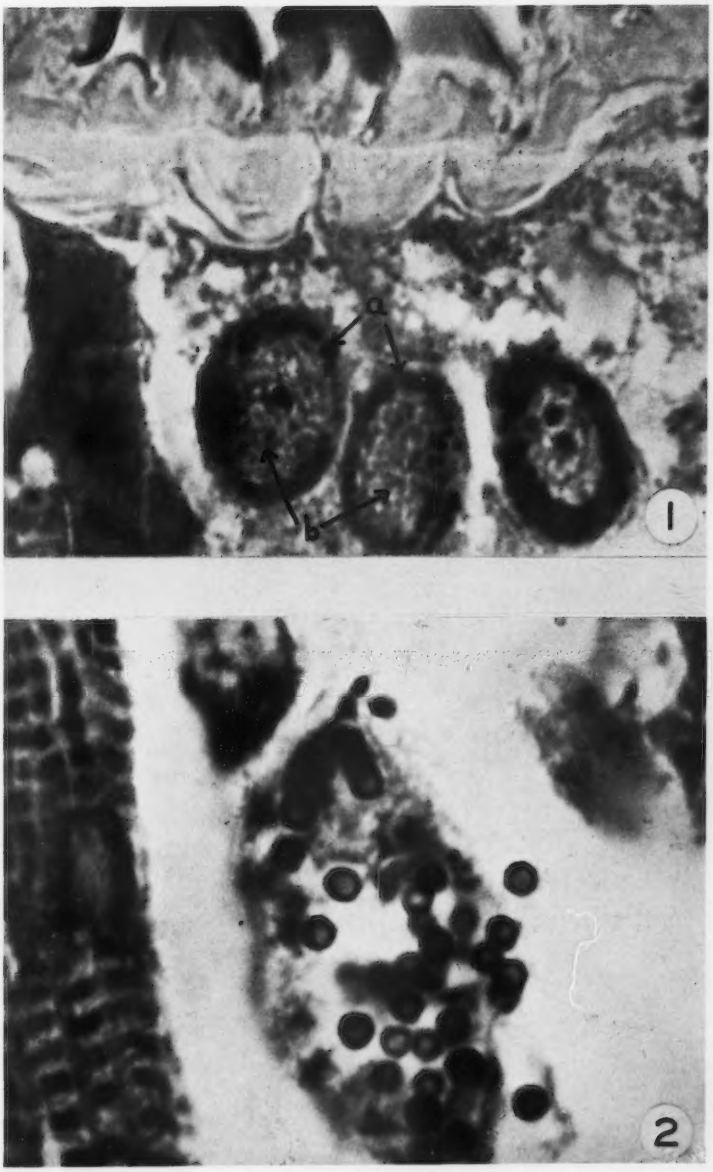
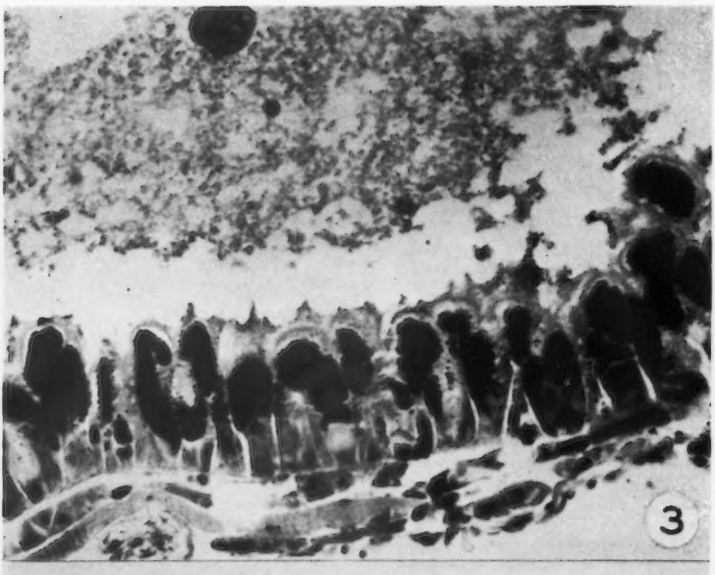



FIG. 1. Section of the hypodermis of an infected spruce budworm larva. Immature polyhedral bodies (*a*) can be seen surrounding the central chromatic mass of the nucleus (*b*). × 2500 FIG. 2. Section of a tracheal matrix cell of an infected spruce budworm larva. Mature polyhedra fill the nucleus. × 2500



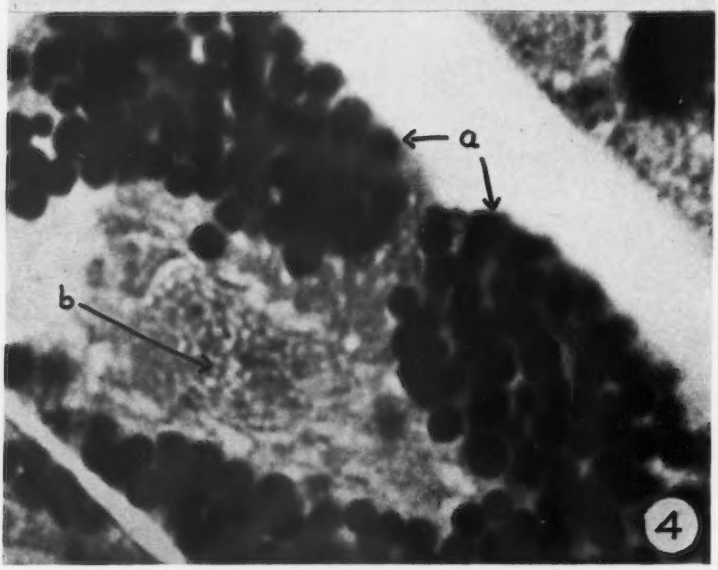




FIG. 3. Section of the mid-gut epithelium of an infected spruce budworm larva showing colonies of polyhedral bodies in the cytoplasm of the cells. × 900 FIG. 4. Section of an epithelial cell from the mid-gut of an infected larva, showing (*a*) masses of polyhedral bodies surrounding (*b*) the nucleus. × 2600

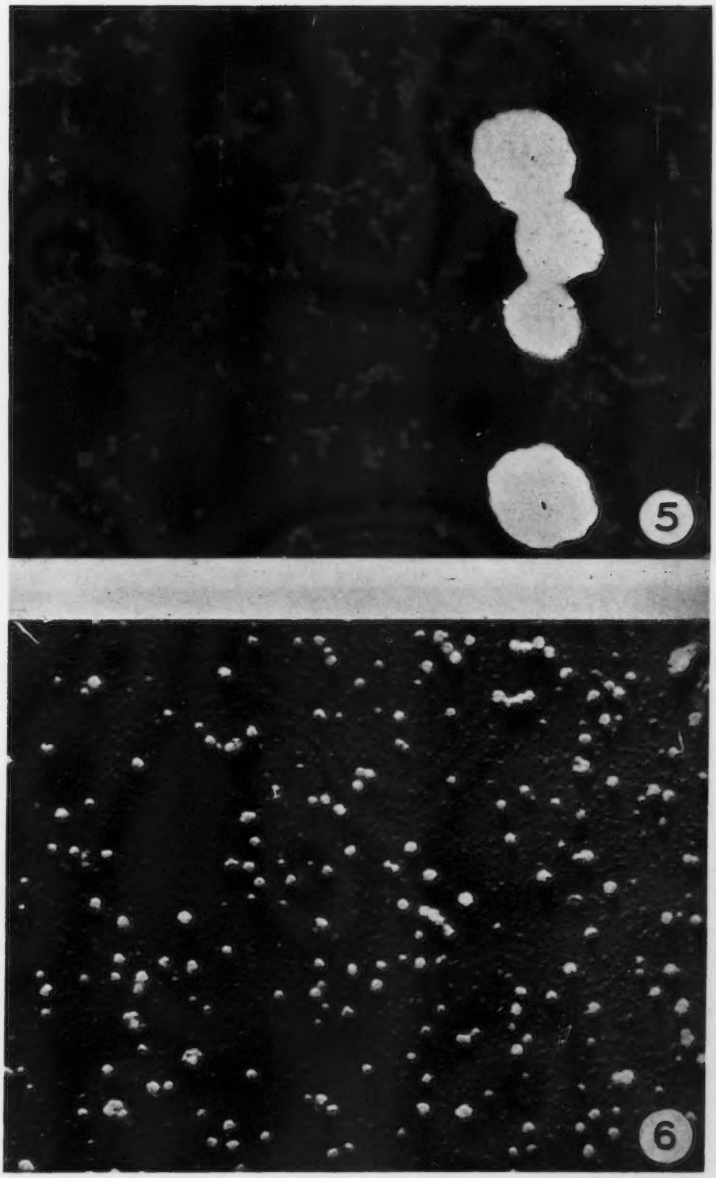



FIG. 5. An electron micrograph of four polyhedra and numerous small spherical particles isolated from a colony of cytoplasmic polyhedra. × 25,000 FIG. 6. An electron micrograph of small spherical particles isolated from cytoplasmic polyhedra dissolved in weak alkali. Uranium shadowed. × 25,000

TABLE I

MORTALITY CAUSED BY FEEDING SPRUCE BUDWORM LARVAE CYTOPLASMIC POLYHEDRA*

| | Number of larvae | Died from cytoplasmic disease | Died from cytoplasmic and other diseases | Died from nuclear disease | Died from other diseases | Survived to adults |
|---|---|---|---|---|---|---|
| Larvae fed cytoplasmic polyhedra | 332 | 39.75% | 24.69% | 0.3% | 13.82% | 21.38% |
| Larvae fed sterile distilled water | 173 | 1.15% | 0.57% | 0% | 28.91% | 69.36% |

** Suspension contained 500 or more colonies of cytoplasmic polyhedra in 0.5 ml. of water. A drop of the suspension was placed on a glass slide and larvae, observed under the microscope, fed for one minute.*

The two diseases have not been found occurring together in nature. An examination of sections from 1088 field-collected spruce budworm larvae showed that 12 of the larvae (1.1%) were infected with the cytoplasmic disease. None of the larvae were infected with the nuclear disease.

## Discussion

Polyhedra of most insect viruses, whether they are formed in the nuclei of tracheal matrix, fat, blood, or hypodermal cells of Lepidoptera (6,8,9,11,13,15) or whether they are formed in the nuclei of mid-gut cells of Hymenoptera (7), contain mostly rod-shaped particles and frequently also spherical particles which develop into rods (3, 5, 7). Smith and Wyckoff (16) observed, however, that polyhedra formed in the nuclei of the cells of certain Lepidoptera contain only spherical particles, demonstrating that two types of polyhedral viruses occur: one in which the polyhedra contain mostly rod-shaped particles and one in which the polyhedra contain only spherical particles. The cytoplasmic polyhedral virus disease of the spruce budworm is of the second type in that the polyhedra contain only spherical particles. It differs from most polyhedral viruses so far described in that polyhedra are formed in the cytoplasm and not in the nucleus of the cell.

The infectious nature of the cytoplasmic polyhedra was repeatedly demonstrated and with few exceptions larvae fed the polyhedra developed the disease within 72 hr. Many larvae, presumably infected with the disease, survived, indicating that the disease is not particularly virulent or that infected mid-gut cells are sloughed off and replaced by new cells, a process which apparently occurs continuously in normal larvae.

The possibility that spruce budworm larvae are susceptible to a single virus which multiplies first in the cytoplasm of mid-gut cells and later in the nuclei of other cells was disproved, not only because the polyhedra contain different types of particles but also because washed cytoplasmic polyhedra, when fed to spruce budworm larvae, produced only the cytoplasmic type of infection.

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# TEMPERATURE-CONTROLLED MERISTIC VARIATION IN THE PARADISE FISH *MACROPODUS OPERCULARIS* (L.)[1]

By C. C. Lindsey[2]


## Abstract

Eggs from one pair of paradise fish *Macropodus opercularis* (L.) were reared at different constant temperatures, and the effects were noted on dorsal and anal fin ray, spine and basal counts, caudal and pectoral ray counts, and vertebral counts. Some young were transferred from low to high temperatures after varying periods to determine the duration of sensitivity of meristic series to environmental influence. Intermediate sustained temperature produced significantly fewer vertebrae than either low or high temperature. Different rearing temperatures produced different ray, spine, and basal counts; variation in each of these series was largely independent of vertebral variation and of variation in the other series. Eggs hatched less than two days after fertilization, but some meristic series were still subject to environmental influence 20 days later. The order of fixation of the final count in different series roughly followed the order of their ontogenetic appearance. While "shock effect" was apparent in some changes produced by temperature transfers, there was a general tendency for counts of certain lots of fish transferred from low to high temperature to approximate counts of fish reared at sustained intermediate temperatures.


## Introduction

The recent review by Tåning (5) of meristic characters in fishes demonstrates the paucity of experimental studies on meristic variation, regarding both the number of species tested and the number of meristic series examined. Those experiments which have been reported indicate a confusing diversity in the effect of given environmental factors on one meristic series in different species, and on different series within one species. Further, almost the only experiments to determine the period of development at which meristic characters are subject to environmental influence have been those of Tåning using *Salmo trutta*. Clearly, many more investigations must be made before generalizations can be attempted regarding the degree and direction of phenotypic plasticity in meristic series of teleosts.

The present contribution concerns the direction and duration of the influence of temperature on the vertebral and fin ray series in the paradise fish *Macropodus opercularis* (L.). This species differs from *S. trutta* in that hatching occurs extremely rapidly (*ca.* 24 hr. at 28° C.) at a relatively early stage of development. As the dorsal and anal fins of the species extend along the greater part of the body, it has also been possible to examine a question which has previously received little attention, that of the degree of dependence of fin ray count on vertebral variation.

## Material and Methods

Air-breathing "labyrinth fishes" (family Anabantidae) of the genus *Macropodus* inhabit fresh waters of south and east Asia, where they comprise "a few

[1] *Manuscript received December 11, 1953.*
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varied but puzzling species" (2). *M. opercularis*, the "paradise fish" of aquarists, is convenient for experimental purposes, as it lays several hundred eggs at one time, and may spawn every three or four weeks. The eggs, which float, are placed by the male in a floating bubble-nest, and the larvae, which hatch rapidly, remain in the nest for some time.

All eggs used in the present experiments came from two successive spawnings of one pair of fish, obtained from the aquarium in the Zoological Laboratory at Cambridge, England, where these experiments were conducted. Each spawning took place over a period of about two hours in an aquarium at 27° C., after which the bubble-nest and eggs were removed and divided into three parts. Each mass of bubbles and eggs was placed within a platform of cheesecloth and cork, floating in a 500 ml. beaker of water at 27°. Each beaker was floated in a constant temperature aquarium maintained within 0.1 C. degrees by electric heaters and mercury–toluol thermostats. Beakers were covered until after hatching. No aeration was provided, but water was changed daily for the first week. Larvae were fed first on infusorian cultures, then on nematode "microworms", and later on newly hatched *Artemia* and on *Daphnia*. Older fish were divided amongst several beakers, and, in the case of the 24° lot, all fish were released, seven weeks after hatching, into an aquarium at 24°. Fish were preserved at from 10 to 20 mm. standard length (8 weeks old at 31°, 13 weeks at 24°), and were later cleared in potassium hydroxide and stained with alizarin.

Experiments were also conducted to determine, by transferring fish from one temperature to another at various stages of development, at what period certain meristic characters become fixed (3). A large number of larvae were hatched at 24°; of these, samples were transferred to separate beakers of 24° water which were then placed in 31° tanks for the remainder of the experiment. Such transfers were made 4, 13, and 22 days after fertilization. In view of the small sample sizes, absence of significant difference between meristic counts of a lot transferred to 31° and the lot reared constantly at 24° does not demonstrate conclusively that the transferred lot was subject to no further environmental influence at time of transfer. However, presence of a significant difference despite small sample size does demonstrate that environmental influence was still effective *at least* as late as the time of transfer.

Accurate determination of the proportion of eggs hatching was difficult, as eggs were not readily distinguishable from nest bubbles, and at high temperatures decomposition of dead eggs was rapid. Probably over 80% of eggs hatched at temperatures from 20° to 31°, while at 32°, which is evidently close to the upper limit of tolerance, only about 40% hatched and none survived. As is common amongst such fishes reared in aquaria, subsequent mortality was high at all temperatures. Optimal temperature for survival and growth appeared to be between 24° and 28°. Table I gives the number of fish surviving in each lot. Meristic counts of these are shown in the Appendix.

TABLE I

NUMBERS OF *M. opercularis* REARED AT DIFFERENT TEMPERATURES

| Temperature, ° C. | Days from fertilization to transfer | Number surviving | Date of spawning, 1952 |
|---|---|---|---|
| 32 | — | 0 | Jan. 18 |
| 31 | — | 10 | Feb. 12 |
| 28 | — | 9 | Jan. 18 |
| 24 | — | 49 | Feb. 12 |
| 20 | — | 0 | Jan. 18 |
| 24 → 31 | 4 | 6 | Feb. 12 |
| 24 → 31 | 13 | 9 | Feb. 12 |
| 24 → 31 | 22 | 9 | Feb. 12 |

## Vertebrae

Fig. 1 is a camera lucida drawing of a juvenile paradise fish treated with potassium hydroxide and alizarin dye. In this species one, two, or three fin rays may lie adjacent to one body segment, and the number of rays and spines of a fin may exceed the total number of vertebrae. The dorsal and anal fins each consist of an anterior series of spines and a posterior series of segmented rays; the distinction between the two is not always clear in young specimens, and the supporting basals are similar. One basal supports each spine or ray, except at the extreme posterior of the anal fin where rays and basals may be "out of step" and one ray may occur without a basal. A variable number of spineless basals lie anterior to the first dorsal spine, but no spineless basals were found interpolated *within* a spine or ray series.

Abdominal vertebrae may readily be distinguished from caudal vertebrae in *M. opercularis* by the long haemal process of the first caudal vertebra

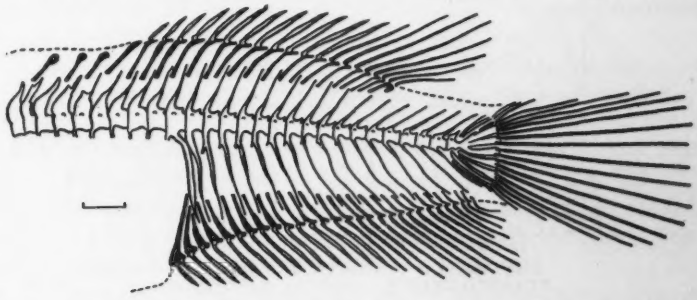

FIG. 1. Vertebral and median fin skeleton of juvenile paradise fish *Macropodus opercularis* reared at 24° C. Scale 1 mm.

(Fig. 1). The two anterior vertebrae are close together but may be distinguished in cleared specimens by their neural processes. The penultimate vertebra is frequently reduced in size; the presence of a distinct centrum was taken as the criterion in counting. The urostyle was included in these counts.

Abdominal and caudal vertebral counts varied from 8 to 10 and from 18 to 20 respectively, with total counts from 27 to 29 (Appendix). Variation in means at sustained and at transferred temperatures are presented graphically in Fig. 2.

In this and subsequent figures the large circle represents the lot of 49 fish reared at a constant temperature of 24°; sample sizes in the remaining lots, as shown in Table I, were only 6 to 10.

Variation in total vertebral count at constant temperatures (Fig. 2) has the same V-shaped form as previously shown for *Salmo trutta* (5) and also found for *Gasterosteus aculeatus* by the author (unpublished). Mean total count is significantly lower at 28° than at either 24° or 31° ($p$ .01–.02 and $p < .01$ respectively). (Statements of probability in this paper are based on $T$-test analysis of the data presented in Appendix. Such procedure is open to some criticism, but is adequate for present purposes with the proviso that caution be exercised in basing any conclusions on statistical comparisons between such small samples.) It may be seen by comparison of the graphs of abdominal and caudal vertebrae that the decrease between 24° and 28° is confined to the caudal region, while from 28° to 31° both abdominal and caudal counts rise.

Results of transfers are given on the right hand graphs in all figures. In each case the extreme right and left hand points indicate means at sustained

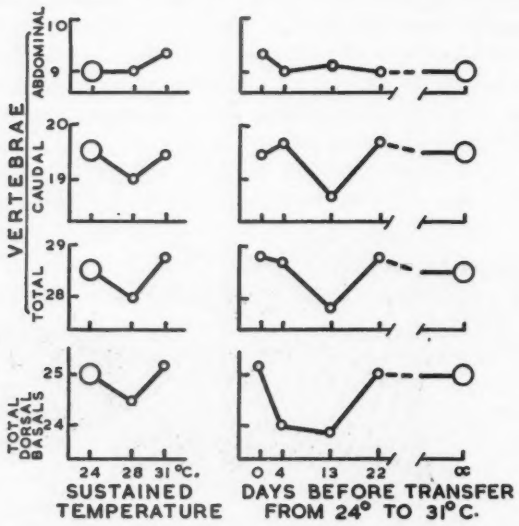


FIG. 2. Influence of temperature on vertebral and total dorsal basal count of *M. opercularis*. For further explanation of this and succeeding figures see text.

temperatures of 24° and 31° respectively, while intermediate points give means for lots transferred from 24° to 31° at given intervals following fertilization. (Hatching at 24° occurs from 30 to 50 hr. after fertilization.)

Possible interpretations of the direction of changes produced in some transfers are discussed later; whatever these may be, the principal value of the transfer experiments reported here is merely to locate the period over which meristic counts are still subject to environmental influence.

As shown in the right hand graph of Fig. 2, abdominal vertebral count appeared to be largely determined within the first four days, approximating the means for 24° in all lots initially subject to this temperature. On the other hand caudal vertebrae, which appear later in ontogeny, are evidently subject to environmental change as late as 13 days after fertilization; the sample transferred from 24° to 31° at this time had a significantly lower mean than samples reared at sustained temperatures of either 24° or 31° ($p < .01$ for total vertebrae). In keeping with the above, abnormalities of the anterior vertebrae were commonest in the lot transferred at four days, while posterior abnormalities (fusions or curvatures) were commonest in the lot transferred at 13 days. (Six of nine individuals in the latter group showed curvature of the last two or three vertebrae, as contrasted with only one and two such abnormalities in lots of nine fish reared at 31° and lots of 49 reared at 24° respectively.)

## Anal Fin

The anal fin series develops anteriorly and posteriorly from a point near the definitive posterior anal spines. The spine series, developing postero-anteriorly, is first to complete its appearance. From three to six of the anterior anal spines lie under the posterior wall of the abdominal cavity anterior to the first haemal process (Fig. 1) and are evidently not associated structually with particular vertebral segments. The basals of the first two or three of these spines are frequently fused (Fig. 1).

Mean anal spine counts of individuals reared at sustained and at transferred temperatures are shown in Fig. 3. Variation in the number of spines is evidently not dependent on axial variation, for unlike the V-type relationship of vertebral count to temperature, anal spine count was intermediate at intermediate temperature and lowest at high temperature. As further evidence of the autonomy of anal spine variation, some (but not all) of this variation was produced by the anterior free end of the series as shown in the graph labelled "prehaemal spines" (i.e. those spines anterior to the haemal process of the first caudal vertebra). Both prehaemal and total anal spine counts were significantly higher at 24° than at either 28° or 31° ($p < .01$).

In keeping with the early appearance of the anal spine series, the definitive number is evidently determined comparatively early, in the period from 13 to 22 days (right hand of Fig. 3).

Anal fin ray count (Fig. 3) shows a V-type relationship to temperature; ray count at 28° is significantly lower than at 24° or 32° ($p < .01$). Unlike the

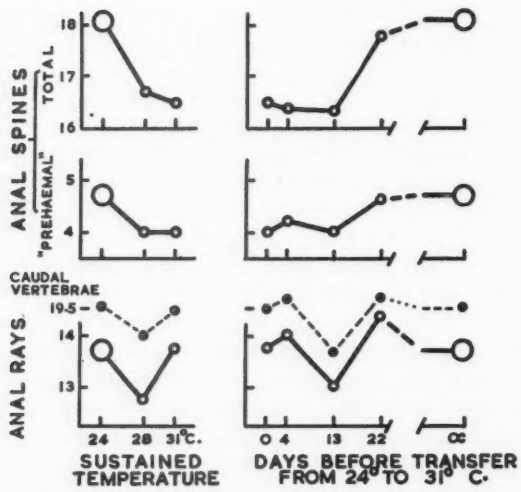


FIG. 3. Influence of temperature on number of anal fin elements of *M. opercularis*.

anal spines, the anal ray series, developing anteroposteriorly, is late in reaching full development. Associated with its late appearance, the definitive ray count may evidently be subject to environmental influence even 22 days after fertilization. A significant decrease in rays, below the mean of the 31° lot, occurred in fish transferred at 13 days ($p < .01$). A change in the reverse direction occurred in those transferred at 22 days, producing a count somewhat higher than that of fish reared constantly at 24° ($p$ .01–.02). The meaning of these changes is obscure, but there is striking correspondence between effects on caudal vertebrae and on anal rays (the latter shown as a broken line for comparison in Fig. 3), despite the fact that definitive vertebral segmentation is visible well before the anal rays have appeared. Although the posterior extension of the anal ray series may be limited by its proximity to the hypural (Fig. 1), there is not a fixed relation between axial and fin segments. One, two, or three anal fin rays may lie adjacent to one vertebra, and the mean number of rays per segment tends to be related inversely to caudal vertebral count (suggesting that, relative to the rays, the vertebral column is in different cases divided into either a few long or many short segments). Hence, the number of anal rays and the number of caudal vertebrae are probably not causally connected, but their reactions to the stimuli here applied were similar.

## Dorsal Fin

In the first formed region of the dorsal series (the posterior dorsal rays), two or even three basals occupy each body segment, while in the later formed anterior region all but one or two segments support a single basal (Fig. 1). At the extreme anterior, one vertebral segment with no corresponding basal is

sometimes interpolated in the spineless basal series. The dorsal basal series, particularly in the anterior half, thus follows vertebral segmentation rather more closely than does the anal. Correspondingly, environmentally induced variation in the total number of dorsal basals (lumping together ray basals, spine basals, and anterior spineless basals given in the Appendix) tends to parallel vertebral variation, as shown in the bottom graphs of Fig. 2. Like total vertebral count, total dorsal basal count is probably largely determined before 22 days.

The order of appearance of dorsal spines and rays is the reverse of the anal fin; dorsal rays appear first (but later than the anal spines), while the postero-anteriorly forming dorsal spines are not all visible until later. The posterior end of the ray series is a considerable distance anterior to the hypural. The anterior end of the spine series is continuous with the spineless anterior basals (Fig. 1).

Unlike the underlying series of basals, the dorsal spine and dorsal ray counts are both relatively insensitive to differences in sustained temperature, as shown in Fig. 4. The number of vertebrae anterior to the first dorsal spine, as indicated by the serial number of the vertebra adjacent to the anterior (i.e. last-formed) spine, is also shown in Fig. 4. From this it appears that whatever may be the factor which limits forward extension of the dorsal spine series, it is probably not proximity to the anterior end of the axis, for in lots having higher vertebral counts, there are on the average more vertebrae anterior to the first dorsal spine. Determination of the final number of parts in the dorsal spine series is, as in previously discussed series, evidently largely independent of spacing of adjacent series.

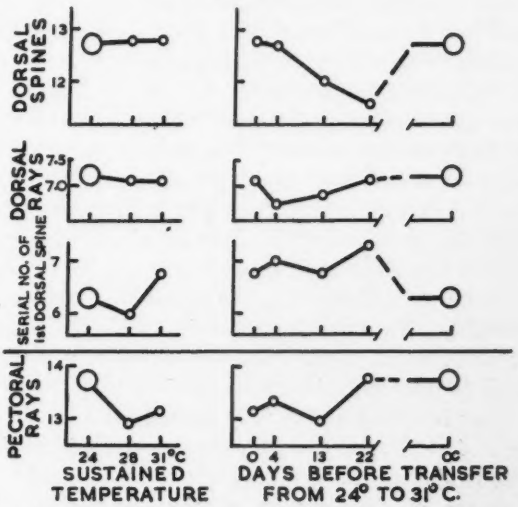


FIG. 4. Influence of temperature on number of dorsal fin elements and pectoral fin rays of *M. opercularis*.

Reactions to temperature transfers again reflect the order of appearance of definitive parts. Dorsal rays, which are formed comparatively early, showed a slight decrease in the lot subject to low temperature for the first four days, but counts were evidently fully determined before 22 days (Fig. 4). The later formed dorsal spines, on the other hand, are evidently still subject to profound modification at this time; the lot transferred from low temperature at 22 days showed spine count markedly lower than the lot reared continuously at low temperature ($p < .01$).

Some information as to the nature of this reduction is available from examination of the serial number of the vertebra opposite the anterior (i.e. last-formed) dorsal spine (Fig. 4). This serial number was significantly higher in the 22-day transfer lot than the continuous low-temperature lot ($p < .01$). Presumably, then, the sharp reduction of spine count in the former was due not to sparser spacing of the spines along the axis, but to suppression of the last-formed spines.

While the possibility of modifications later than 22 days cannot be ruled out, the present data suggest that the anterior basals, although becoming visible (in stained specimens) later than the spines, are in fact determined at an earlier period. The number of basals is apparently largely fixed before 22 days after fertilization, while the number of these which will carry spines is still subject to sharp environmental inhibition.

## Caudal Rays

The caudal fin rays (following the nomenclature of Ginsburg (1) ) consist of long "segmented" or "jointed" rays rising from the hypural (which initially appears as a number of separate lobes), and of a dorsal and ventral series of shorter simple unsegmented rays (Fig. 1). Distinction between the two types is, however, not always clear, as joints may develop in the outer rays after the fish has reached considerable size. The dorsal and ventral hypural halves bear from five to seven and from seven to nine segmented rays respectively, with from two to four and one to three simple rays associated with them. There was suggestion in the material examined of a tendency towards fewer caudal rays at high temperature (a phenomenon also found by the author in *Gasterosteus aculeatus* (unpublished) ), but trends were obscured by a curious form of abnormality. In some fish an additional set of (usually six) full-length rays occurred dorsal to the usual segmented rays, producing in effect an additional tail fin interpolated between the dorsal segmented and the dorsal simple rays. Four individuals with fully formed extra caudal lobes occurred amongst fish reared at 24°, and two amongst those transferred from 24° in four days. Other individuals with high numbers of dorsal caudal rays suggested intermediate stages of development of the peculiarity. No associated duplication of hypural elements was noted.

## Pectoral Rays

The pectoral fins bear from 10 to 14 (usually 13 or 14) rays (the uppermost of which is shorter and structurally dissimilar to the remainder). Rays were counted on both sides on the majority of fishes (Appendix). Pectoral ray count is subject to environmental variation, decreasing significantly at temperatures above 24° as shown in the bottom graph of Fig. 4 ($p < .01$). Significant rise in mean between 28° and 31° cannot be shown with the present data. Determination appeared to be largely complete before 22 days at 24°.

## Discussion

Observed meristic variations in the paradise fish have been assumed to be attributable to direct environmental influence on the development of individuals, rather than to genetic selection. Alternatively, as mortalities were high, these variations might be attributed to differential survival, at different temperatures, of genotypes each producing specific meristic counts. However, with many characters varying independently at different sustained temperatures, and with complex and unrelated changes occurring in lots transferred between temperatures, it would be necessary to postulate a most unwieldy complex of genes to cover all the observed facts. Temperature may well have had some selective action, but direct influence on ontogeny probably accounts for most of the meristic variation between experimental lots. Such a hypothesis is consistent with experiments on other species in which mortality was relatively low and in which meristic variation was almost certainly due to phenotypic plasticity.

Different meristic series which have been examined in the paradise fish show different reactions to sustained and also to transferred temperatures. While some association occurs between variation in certain meristic series, each median fin series evidently has its own pattern of variation, in some cases quite independent of that of structurally related series.

The mechanics of environmental determination of meristic counts are not at present understood, but the lack of correspondence between variation in different series suggests that different mechanisms may be operative in different meristic series of the same species. Moreover, V-shaped curves suggest that some meristic counts may represent the resultant of two mechanisms reacting to temperature in opposite directions.

Some series have been shown to be susceptible to environmental influence as long as 20 days after hatching. Although Tåning (5) notes that the numbers of vertebrae in several temperate zone fishes are evidently determined before hatching, emergence from the egg is evidently no criterion for cessation of environmental influence in the rapidly-hatching tropical species here discussed. The comparatively simple mechanism of temperature-controlled rate of diffusion of oxygen through the chorion of the developing egg cannot in this case be the factor producing meristic differences, for the fish are hatched and free swimming throughout much of their temperature-sensitive period.

In keeping with findings for other species, the periods of fixation of different meristic characters in *Macropodus* have been found in general to reflect the order of their visible appearance in ontogeny.

Transfers of developing fish from one temperature to another have produced ambiguous results. Tåning (4) demonstrated marked changes in vertebral count of *Salmo trutta* by subjecting eggs briefly to altered temperatures during a short critical period which directly precedes final determination of vertebral count. Tåning has called this phenomenon "shock effect". The change induced is in the opposite direction to that produced by sustained rearing at the shocking temperature.

Some of the alterations produced in the present instance by temperature transfers probably fall in the category of shock effects. The marked reduction in dorsal spines in 13- and 22-day transfers has this appearance, as the counts thus produced were much lower than counts for any of the three sustained temperatures. This reduction in spines (but not of their basals) suggests that in this series shock effect involved suppression of the latest forming elements of the series. Such cannot be true, however, of all effects grouped under this title. Shock effects are probably at least as complex and varied, in different meristic series, as the mechanisms governing the effects of sustained temperatures.

On the other hand, behavior of vertebral, total dorsal basal, and anal ray counts showed a certain regularity in their reactions. Concomitant with the V-shaped curves of counts against sustained temperature, the curves showing counts for fish transferred from low to high temperature after various intervals were also V-shaped. Temperature effects in the lot transferred from low to high temperature after 13 days were apparently similar to the effects of sustained rearing at an intermediate temperature. The fact that the absolute value in the transferred lot did not necessarily correspond to the value for the lot reared at sustained intermediate temperature might be simply a reflection of the fact that somewhat lower counts would have been achieved at sustained temperatures slightly higher or slightly lower than the intermediate chosen.

Not all the observed phenomena of temperature transfers fall in the above category; for example, anal ray count in the 22-day transfer lot was *higher* than counts for either high or low temperature. Metabolic upset resulting from transfer at a critical period very probably is responsible for some of the results obtained. Nevertheless, it is notable that characters with V-shaped sustained temperature curves also showed a minimum in lots transferred between the extreme temperatures, while anal spine count, with a simple inverse relation to sustained temperature, showed no marked depression in transferred lots.

Small sample sizes preclude any but tentative conclusions from the present data. Clearly elucidation of the details of meristic variations, even in a single species, will require a much more extended series of experiments, using smaller temperature intervals and more frequent transfers.

## Acknowledgments

It is a pleasure to acknowledge the assistance of Prof. James Gray, who supervised this study, and of Mr. Kenneth Klose, and Dr. H. W. Lissmann, all of the Cambridge Zoological Laboratory. Mr. T. G. Northcote and Dr. P. A. Larkin, both of the British Columbia Game Department, kindly criticized the manuscript. Financial assistance was provided by the Research Council of Ontario.

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## Appendix

### MERISTIC COUNTS AT SUSTAINED AND TRANSFERRED TEMPERATURES, *M. opercularis*

SUSTAINED TEMPERATURES ARE AS INDICATED; TRANSFERS FROM 24° C. TO 31° C. ARE INDICATED BY *T* FOLLOWED BY THE NUMBER OF DAYS FROM FERTILIZATION BEFORE TRANSFER. ALL FISH ARE FROM SAME PARENTS

| | Total vertebrae | | | | Abdominal vertebrae | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 27 | 28 | 29 | Mean | | 8 | 9 | 10 | Mean |
| 31° | | 2 | 7 | 28.78 | 31° | | 6 | 3 | 9.33 |
| 28° | | 9 | | 28.00 | 28° | | 9 | | 9.00 |
| 24° | 2 | 21 | 26 | 28.49 | 24° | 2 | 46 | 1 | 8.98 |
| *T*4 | | 2 | 4 | 28.67 | *T*4 | | 6 | | 9.00 |
| *T*13 | 3 | 5 | 1 | 27.78 | *T*13 | | 8 | 1 | 9.11 |
| *T*22 | | 2 | 7 | 28.78 | *T*22 | | 10 | | 9.00 |

| | Anterior spineless basals | | | | | Anal spines | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | 5 | 6 | 7 | Mean | | 15 | 16 | 17 | 18 | 19 | 20 | 21 | Mean |
| 31° | 1 | 5 | 3 | | 5.22 | 31° | 2 | 3 | 2 | 2 | | | | 16.44 |
| 28° | 5 | 3 | 1 | | 4.55 | 28° | 2 | 1 | 4 | 2 | | | | 16.66 |
| 24° | 12 | 22 | 12 | 3 | 5.12 | 24° | | 4 | 10 | 20 | 8 | 5 | 1 | 18.05 |
| *T*4 | 3 | 2 | 1 | | 4.67 | *T*4 | 1 | 2 | 3 | | | | | 16.33 |
| *T*13 | 2 | 3 | 2 | | 5.00 | *T*13 | 1 | 3 | 3 | | | | | 16.29 |
| *T*22 | | | 7 | 2 | 6.22 | *T*22 | | 1 | 2 | 5 | – | 1 | | 17.78 |

## Appendix—*Concl'd.*

MERISTIC COUNTS AT SUSTAINED AND TRANSFERRED TEMPERATURES, *M. opercularis*—Concl'd.

SUSTAINED TEMPERATURES ARE AS INDICATED; TRANSFERS FROM 24° C. TO 31° C. ARE INDICATED BY *T* FOLLOWED BY THE NUMBER OF DAYS FROM FERTILIZATION BEFORE TRANSFER. ALL FISH ARE FROM SAME PARENTS—*Concl'd.*

| | "Prehaemal" anal spines | | | | |
|---|---|---|---|---|---|
| | 3 | 4 | 5 | 6 | Mean |
| 31° | 1 | 7 | 1 | | 4.00 |
| 28° | 1 | 7 | 1 | | 4.00 |
| 24° | 1 | 15 | 30 | 2 | 4.69 |
| *T*4 | 1 | 3 | 2 | | 4.17 |
| *T*13 | 2 | 5 | 2 | | 4.00 |
| *T*22 | | 4 | 6 | | 4.60 |

| | Anal fin rays | | | | | |
|---|---|---|---|---|---|---|
| | 12 | 13 | 14 | 15 | 16 | Mean |
| 31° | | 2 | 7 | | | 13.78 |
| 28° | 4 | 3 | 2 | | | 12.78 |
| 24° | 2 | 14 | 31 | 1 | 1 | 13.69 |
| *T*4 | | 2 | 2 | 2 | | 14.00 |
| *T*13 | 1 | 5 | 1 | | | 13.00 |
| *T*22 | | | 6 | 3 | | 14.33 |

| | Dorsal spines | | | | | | |
|---|---|---|---|---|---|---|---|
| | 10 | 11 | 12 | 13 | 14 | 15 | Mean |
| 31° | | | 5 | 2 | 1 | 1 | 12.78 |
| 28° | | | 2 | 7 | | | 12.78 |
| 24° | 1 | 1 | 12 | 31 | 4 | | 12.73 |
| *T*4 | | | 2 | 4 | | | 12.67 |
| *T*13 | | 2 | 3 | 2 | | | 12.00 |
| *T*22 | | 4 | 6 | | | | 11.60 |

| | Serial No., 1st dorsal spine | | | | |
|---|---|---|---|---|---|
| | 5 | 6 | 7 | 8 | Mean |
| 31° | | 2 | 7 | | 6.78 |
| 28° | | 9 | | | 6.00 |
| 24° | | 37 | 11 | 1 | 6.27 |
| *T*4 | | | 6 | | 7.00 |
| *T*13 | | 2 | 6 | | 6.75 |
| *T*22 | | | 7 | 3 | 7.30 |

| | Dorsal fin rays | | | |
|---|---|---|---|---|
| | 6 | 7 | 8 | Mean |
| 31° | | 8 | 1 | 7.11 |
| 28° | 1 | 6 | 2 | 7.11 |
| 24° | 3 | 34 | 12 | 7.18 |
| *T*4 | 2 | 4 | | 6.67 |
| *T*13 | 1 | 6 | | 6.86 |
| *T*22 | 1 | 6 | 2 | 7.11 |

| | Pectoral fin rays* | | | | | | |
|---|---|---|---|---|---|---|---|
| | 12 | 12½ | 13 | 13½ | 14 | 15 | Mean |
| 31° | | | 15 | – | 3 | | 13.17 |
| 28° | 1 | 2 | 15 | | | | 12.89 |
| 24° | | | 21 | 1 | 47 | 2 | 13.73 |
| *T*4 | | | 5 | 1 | 2 | | 13.31 |
| *T*13 | 1 | – | 9 | 1 | | | 12.95 |
| *T*22 | | | 5 | – | 10 | 1 | 13.75 |

* *Half rays are those lacking one of the two opposing lepidotrichia halves.*

# OBSERVATIONS ON THE BIONOMICS OF SOME NORTHERN SPECIES OF SIMULIIDAE (DIPTERA)[1]

By B. Hocking[2] and L. R. Pickering[3]

## Abstract

Nine new records of black flies bring the total number of species found in northern Manitoba to 21. Observations on the bionomics, especially of *Simulium venustum* Say, are given. The attachment of black-fly larvae is determined by a mechanical factor, probably the water velocity gradient, perhaps conditioned by visual stimuli. Young larvae were heavily preyed upon by *Hydra* sp. and adults by the white-crowned sparrow *Zonotrichia leucophrys*. Mermithid worms persisted as parasites in the pupal and adult stages. A method is described by which specimens of several species were reared individually in the field from half-grown larvae to the adults, and the exuviae retained. A number of laboratory rearing techniques were tried for *S. venustum* but few larvae molted from the first to the second instar. Adults did not feed on blood or lay eggs in the laboratory, and there was no evidence of successful mating. The difficulties in laboratory rearing of larvae are thought to be due to a sensitivity to autointoxication. The energy relationships of black flies are discussed briefly.

## Introduction

A description of the terrain and weather conditions and a preliminary account of the biting flies at Churchill, Man., was published in 1948 (16). More detailed accounts of the mosquitoes and the tabanids of this area were published in 1950 and 1951 respectively (7, 9). This paper gives further details on the black flies. Most of these observations were made in 1951 or 1952 near Warkworth and Goose creeks, tributaries of the Churchill River, in the forest about 12 miles south of Churchill. The studies were directed primarily at accumulating information of use in attempting the laboratory rearing of *Simulium venustum* Say.

No black-fly species has been reared in the laboratory throughout its life cycle. Dalmat (1) attempted colonization by diverting a stream through a concrete trough in a cage, and Mackerras and Mackerras (8), among others, reared adults from larvae. The latter authors used an aerator based on a filter pump, and reared *Simulium ornatipes* Skuse from egg to adult in this manner. Both of these approaches were used in this study. Eggs and young living larvae are difficult to identify; unless otherwise stated, it is believed that observations on these stages relate to *S. venustum*.

The season of 1951 was early and 1952 was very early. The date on which the ice broke up in the Churchill River, a useful criterion of temperature conditions, was June 11 in 1951 and June 3 in 1952; the average date is June 15.

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## Field Observations

*Species Present*

Since 1948, when Twinn *et al.* recorded 12 simuliids at Churchill, nine other species have been taken there or elsewhere in northern Manitoba, bringing the total to 21 species representing four genera; one of these is undescribed.

There are two species of *Prosimulium:*

*P. gibsoni* Twinn is much the commoner species, and apparently overwinters in the larval stage. Pupae were collected from several streams from June 15 to 19 in 1951 and on June 14 in 1952. Adults were reared from these in the laboratory in 1951, males emerging from June 18 and females from June 21 onward.

*P. decemarticulatum* Twinn is rather rare. A single female was reared from a larva collected in Warkworth Creek in 1951 (Table III).

There are seven species of *Cnephia,* all found near Churchill, and one of them undescribed:

*C. eremites* Shewell (= *Eusimulium* species *A* of Twinn *et al.*, 1948) is common in the tundra streams flowing north into Hudson Bay; pupae were collected on June 29 in 1951 and June 19 in 1952. It has not been taken from the forest streams.

*C. mutata* (Mall.) is fairly common in the forest streams. In 1951 pupae were collected on June 15 and 16; male adults emerged from these on June 20 and females on June 21. In 1952 pupae were collected from June 4 to 11; the first males and females emerged on June 12.

*C. dacotensis* (D. & S.) is locally common. A few larvae were collected early in 1952 and adult males were taken on July 2, 1951. The species was not reared.

*Cnephia* species *N* is rather rare. A few pupae were collected from Warkworth Creek on June 20, 1951; from the Churchill River, C. R. Twinn, Veterinary and Medical Entomology Unit, Ottawa, collected specimens on July 9, 1947.

The remaining three species of *Cnephia* have been reported by other authors in material collected as follows:

*C. taeniatifrons* (End.), adult ♀ ♀, June 28 and 30, 1948; and *C. sommermanae* Stone ? (= *Eusimulium* species *C* of Twinn *et al.*, 1948; of *C. borealis* (Mall.) group), adult ♀, by Shewell (in litt., 1952, 1953). *C. saileri* Stone, pupae, June 29 and July 1, 1948, by Stone (13).

All of the six species of *Eusimulium* are recorded from Churchill:

*E. furculatum* Shewell (= *Eusimulium* species *B* of Twinn *et al.*, 1948) is the most abundant species breeding in the forest streams. Pupae were collected from June 18 to August 6 in 1951 and from June 7 onward in 1952. Both male and female adults emerged on June 25 in 1951. In 1952 males were taken on June 16 and females on June 18; empty pupal skins were found on June 14.

*E. latipes* (Mg.) breeds in small numbers in most of the slower forest streams. Pupae were collected from June 21 onward in 1951, and from June 10 in 1952. Adult males emerged in the laboratory on June 21 in 1951 and females on June 13 in 1952.

*E. baffinense* (Twinn) is locally common. Many pupae were collected from the head waters of Goose Creek on July 20, 1951.

*E. aureum* (Fries) is a fairly common species; L. A. Miller, Entomology Division, Ottawa, now Chatham, Ont., reared adult males from pupae collected from a forest stream on July 26, 1948. A mating pair was taken near Warkworth Creek on July 17, 1948. A male was taken on July 2, 1951, and a female on July 20, 1952.

*E. subexcisum* (Edw.) is rather rare at Churchill. Pupae and an adult male were collected on June 18, 1951. A pupa was collected on June 13, 1952, and a female emerged from it on June 15.

In addition to these species, Shewell (in litt., 1953) has recorded *E. pugetense* D. & S.

The six species of *Simulium* are as follows:

*Simulium venustum* Say is the only important biting species at Churchill. It is abundant, breeding in nearly all streams and rivers. In 1951 the first pupae were collected on June 18, the first adults, male and female, on June 23. In 1952 pupae were taken on June 7; adults were not taken until June 18, but empty pupal skins were found on June 14.

*S. vittatum* Zett. breeds mainly in the Churchill River; a few pupae were found in Warkworth Creek. It apparently passes the winter under the ice since adults were usually taken a day or so after the ice went out of the Churchill River. This species is much more abundant in some years than in others. During the evenings of June 26 to 30, 1952, although not biting, it was the most troublesome species, dense clouds of females beating at the face and getting in the eyes, nose, and mouth.

*S. decorum* Wlk. is a rather rare species. The first adult black flies taken in 1951, on June 14, were of this species although at that time pupae had not been found locally. Pupae were collected from June 18 to 21.

*S. arcticum* Mall. has not been taken in the immature stages at Churchill. Adults were collected by Heydweiller on July 30, 1934, and by McClure from July 11 to 23, 1936 (16). Shewell (in litt., 1952) stated that he has a specimen from Baker Lake, N.W.T. On Aug. 10, 1951, many pupae were collected at Mafeking, Man., 450 miles southwest of Churchill. Immature stages do not appear to have been collected any nearer Churchill than this. This species is known to have a long flight range (11).

*S. perissum* D. & S. was identified by Shewell (in litt., 1953) in adult female material collected at Churchill by Twinn on July 6, 1947, but it has apparently not been recorded there since.

*S. luggeri* N. & M. has not been taken at Churchill, but pupae from which adults were reared were collected at Swan River, Man., 490 miles southwest on Aug. 11, 1951.

*Eggs*

In 1951 the search for eggs was begun early in June, as soon as open water appeared in the streams. The water temperature at this time was 32–34° F. The water levels dropped rapidly. Careful search in places where oviposition had been recorded in previous years yielded no egg masses. On June 9, when larvae were beginning to appear, attention was transferred to the beds of the streams, since Fredeen *et al.* (3) had found eggs of *S. arcticum* in this situation. A number of samples of stream bottom material were collected from Goose Creek and another infested stream, and examined by brine flotation and filtration through bolting silk. One empty shell, of the same size as the eggs of *S. venustum*, was found, but no living eggs. Twinn (in litt., 1952) found eggs attached to the concrete walls of a dam at Churchill, just above the water line, on May 31, 1948; these were placed on wet cotton and hatched on June 7.

Eggs of the next generation were collected in the field on July 9, 1948, and hatched in the laboratory on the same day. In 1951 the first eggs of the second generation were found in masses on grasses, willow leaves, and a filamentous alga, *Cladophora* sp., along the north bank of Warkworth Creek on July 6. Further batches of eggs were laid on July 8, 9, 14, 16, and 18, and on Aug. 4, 11, and 12. In 1952 the first eggs found were laid on June 30. The average dimensions of eggs from these batches were 0.25 × 0.18 mm. These compare with previous measurements of *S. venustum* eggs of 0.26 × 0.18 mm. (2) and 0.23 × 0.14 mm. (17).

Eggs laid on the evening of July 8 hatched in the laboratory on July 17, 171 degree-days above 32° F. Eggs laid on the evening of July 16 hatched in the laboratory on the morning of July 24, 174 degree-days; other eggs from this batch had already hatched in the field. The darkening in color as the eggs mature occurs partly in the chorion and partly in the embryonic head. A well developed hatching spine on the vertex and a broad area around it take on a deep blue–black coloration and the two eyespots on either side of the head become heavily pigmented. The head fits closely into the anterior and most acute angle of the egg. The anterior end splits off at hatching. The appearance of the split, which originates above the hatching spine, is immediately preceded by peristaltic waves of the abdominal wall and rotary movements of the head. In the laboratory, about 20 min. is required from the appearance of the split until the larva is free.

Two batches of eggs were found that failed to develop. One batch of 404 eggs appeared freshly laid when found in Warkworth Creek on July 14 and showed no development by July 26. The second batch, of 613 eggs, was found on July 24 among other batches in advanced stages of development.

Batches of eggs laid on Aug. 12, 1951, were stored on ice. Portions of these were placed in snow water at room temperature on March 1, 10, 20, and 30, 1952, but no eggs hatched.

On Aug. 10, 1951, a batch of 10 empty shells, of the same size as the eggs of *S. venustum*, was collected on a cocoon of *S. vittatum* from the Churchill River.

*Larvae and Pupae*

In 1951 the first larvae of the season were found on June 6 in a small stream just below an old dam. They were few in number, varied in size, but were mostly in the first instar, and were attached to the undersides of vegetation close to the bottom of the stream. The larvae released their attachments readily, especially the smaller individuals. The water temperature was 44° F.

By June 8 larvae were present in small numbers in most of the streams, in some drainage ditches, and in Goose Creek. By June 14 they were abundant in practically all running water, but with marked variation in size. Many of the larger specimens were of *Prosimulium* spp.; later observations indicated that the smaller ones were mainly of *S. venustum* and *E. furculatum.*

A distinctive pattern in the arrangement of larvae on rocks and especially on vegetation has often been observed in places where the water flow is laminar. A preliminary investigation in 1951 indicated that larvae were more numerous on the vertical and upstream faces of submerged rocks than on the horizontal faces, and that an upright blade of grass or sedge carried more larvae per unit of its length than one strongly deflected by the current. In 1952, a more specific study was made in a uniform stretch of stream in which the arrangement pattern was well shown and in which grasses rooted in the bottom of the stream provided the main attachment sites. Blades of grass, or parts of blades, were selected with approximate inclinations to the direction of the current of 10°, 45°, and 70° and were marked by notching with scissors. Total lengths of 1 meter of grass blade were marked for each inclination, and the total number of larvae on each meter was counted. The results (Table I) indicate that on linear attachment sites the number of larvae per unit of length was approximately proportional to the sine of the angle of inclination to the current. Although no measurements were made, this appears to be true also for broad attachment surfaces.

In 1951 very young larvae became progressively fewer from about June 16. The latest date on which first-instar larvae of this generation were found was June 22. By July 5 no larvae less than one-quarter grown were found, and by July 15 the only remaining larvae were nearly full-grown. The first larvae of the second generation were found on July 18. Their numbers increased over

TABLE I

RELATIONSHIP BETWEEN INCLINATION OF GRASSES TO THE CURRENT DIRECTION AND DENSITY OF BLACK-FLY LARVAE ATTACHED TO THEM, CHURCHILL, MAN., 1952

| Inclination, $\theta$ | No. of larvae on 1 m. of grass | Sin $\theta$ | Sin $\theta$/larvae per cm. |
|---|---|---|---|
| 10° | 60 | 0.1737 | 0.290 |
| 45° | 230 | 0.7071 | 0.307 |
| 70° | 310 | 0.9397 | 0.302 |

the next two weeks, but they did not become as numerous as expected from the abundant egg masses, although nearly all of the eggs in these hatched. In 1952 there were two broods in the first generation. By June 4 the first brood was already half-grown, and the water was very low in the streams, some of which had dried up completely. Several inches of rain fell during the next week, causing a second spate. A second brood of black-fly larvae hatched at this time. On June 13, streams that had been dry on June 4 contained large populations of very young larvae. These larvae matured rapidly, and although the first eggs were laid a week earlier than in 1951, there was a more definite break between the two generations; for nearly a week in the middle of July it was difficult to find larvae of either generation.

There was a remarkable difference in the species composition of the populations in Goose Creek and Warkworth Creek. Throughout the season, both in 1951 and 1952, *S. venustum* was the most abundant species in Goose Creek and *E. furculatum* in Warkworth Creek, the average percentages being 81 and 76 respectively. The only big difference between the two creeks is in their origins: Warkworth Creek rises from a shallow lake and Goose Creek from a swamp. In the two creeks there was a similar sequence of vegetation: early in the season *Myriophyllum exalbescens* Fern., with patches of *Sparganium hyperboreum* Laestad., was the most important plant. Early in July a profuse growth of *Cladophora* sp. covered the rocks and stones for a brief period, disappearing almost completely after little more than a week. In 1952 there was a second crop of this alga early in August. Late in the season, the place of *M. exalbescens* was taken by *Potamogeton filiformis* var. *borealis* (Raf.) St. John. Except for the alga, all of these plants were important attachment sites for black-fly larvae and pupae.

Warkworth Creek flows toward the northwest, the direction from which the prevailing wind blows at this time of year. Strong northwest winds back up the water in the shallow lake from which the creek rises, causing a pronounced drop in the water level in the creek and a greatly reduced flow. Although on occasions there was a drop in level of several inches in an hour or two, there was no evidence that larvae were ever stranded; they apparently either moved downward by "looping" or released and reattached at a lower level. When the water returned to its former level, there was no significant movement of larvae back to their previous stations; larvae were then found only in the deeper parts of the creek. On July 14 pupae that had been exposed to the air for two days had contracted, turned very dark, and did not respond to stimuli. Rocks carrying many hundreds of such pupae were wetted and taken into the laboratory, where a few flies did emerge. There had been a little rain during the two days, and the rocks were coated with algae. Many adults emerged from pupae on similar rocks that had only been exposed for 24 hr.

The locomotory and feeding movements of *S. venustum* larvae agree with those reported for British species by Smart (12). In climbing back upstream along a silken release thread, the larva does not use the posterior hooks; the

greatest speed recorded for this process was 2.5 cm. per min. The larval head capsule is strongly hydrofuge, and once this capsule breaks through the surface of the water, the larva is held a prisoner by surface tension. Even large larvae are securely held in this way, but resume their activities at once if they are submerged. When larvae are disturbed, the flick closures of the mouth fans, which presumably are associated with the transfer of food to the mouth, are performed more frequently.

*Adults*

*Mating*

In 1951 two small mating swarms of *E. furculatum* were seen at 1730 hr. on June 25, the first day on which this species was taken. These were formed just to leeward of the tops of tamaracks, 10 ft. high. The temperature was 73° F., relative humidity 25%, wind east at 9 m.p.h., and barometer steady at 29.66 in.; the flies were facing into the wind. About 100 males and 6 females were taken; many of the males had emerged so recently that they still had blisters of blood in the wings. Three hours later on the same evening a larger swarm with the appearance of a mating swarm was seen over the head of an entomologist who was walking south along the railway track. About 100 specimens were taken, of which one was a male and the rest females of *S. vittatum*. The temperature at this time had fallen to 63.5° F., and the relative humidity had risen to 48.5%. Most of the females were heavily engorged with nectar. Further swarms of *E. furculatum* were seen at 1600 hr. on July 8, between the projecting tops of willows along the creek side, and continued for about 30 min. The temperature was 63.5° F., relative humidity 58%, wind northwest at 11 m.p.h., and barometer rising at 29.39 in. The flight characteristics of these black-fly mating swarms were similar to those of mosquitoes, but the swarms were smaller. On July 2 many males of *C. dacotensis* were seen crawling on the rocks just above water level; later in the day and on the following day mating pairs of this species and of *E. furculatum* and *S. vittatum* were collected. A few females of *S. venustum* were found in mating swarms of *E. furculatum*, but although they were sought at all hours of the day and night neither swarms nor mating pairs of *S. venustum* were found. Twenty-three females of *S. venustum* which were attracted to man in the field in July, 1952, however, all had the spermathecae full. Further swarms or mating pairs of *E. furculatum* were observed on July 7, 10, and 23.

*Feeding*

Black flies frequently visit and apparently feed on the nectar of flowers (Table II). The concentrations and quantities of nectar found in the crops of adults of *S. venustum* and *S. vittatum* have been reported elsewhere (6). In 1951 the first black fly feeding on blood was recorded on June 18; the first identified as of *S. venustum* was observed on June 29. By the beginning of July this species was biting man and dogs freely whenever weather conditions were suitable. Flies took from three and one-half to five minutes to feed to

TABLE II

BLACK FLIES VISITING FLOWERS AT CHURCHILL, MAN., 1951 AND 1952

| Black-fly species | Flower species | Date | Nectar in flowers |
|---|---|---|---|
| 1951 | | | |
| *E. furculatum* ♂ and ♀ | *Salix cordifolia* Pursh ♂ and ♀ | June 28–29 | Plentiful |
| *S. venustum*, *E. furculatum* ♂ and ♀ | *Rubus acaulis* Michx. | June 29 | Plentiful |
| *S. venustum* ♀ | *Heracleum maximum* Bartr. | July 26 | Very little |
| *S. venustum*, *E. furculatum* ♂ and ♀ | *Achillea Millefolium* L. | July 29 | Very little |
| *S. venustum* ♀ | *Cicuta Mackenzieana* Raup | Aug. 4 | Very little |
| 1952 | | | |
| *S. venustum* ♀ | *Ledum groenlandicum* Oeder | July 15 | Plentiful |

repletion on man; penetration through the skin of a dog took less time than through that of a man, but blood did not appear to be obtained more rapidly. Attempts to feed adults on bird blood are described in the section on parasites and predators.

*Oviposition*

A series of artificial oviposition sites were set up in Warkworth Creek on July 6, 1951. These consisted of batches of grasses arranged horizontally in the shallows of the creek, with the upstream ends held in place by rocks or fixed to a board with wire staples. The first batches of eggs were found on the grasses at 0930 hr. on July 9. There were no eggs at 1730 hr. on July 8, so that oviposition took place between these two times. At 0900 hr. on July 16 recently laid eggs were again found on one of the artificial sites, and at 1930 hr. on the same day a large number of flies were seen ovipositing. The weather was cloudy and windy but it had been warm and sunny all day. Egg-laying flies were so numerous that they were lined up closely side by side, while others crawled over them seeking a place. Fifty specimens of *S. venustum* were collected. Some of these flies were dissected and the numbers of eggs estimated by weighing the entire ovaries and weighing and counting the eggs in an aliquot part. The number of eggs ranged from 325 to 1050, the average being 594. Of nine such flies, seven showed evidence of having had a blood meal; there was no evidence of this in the other two, both of which contained rather small numbers of eggs. Oviposition was again observed on July 18, at 2000 hr. It appears likely that it always takes place during the evenings of warm days, particularly when these follow a period of inclement weather.

In 1952 artificial egg-laying sites were set up on June 27 and eggs were laid on them on June 30.

*Parasites and Predators*

In 1952 a very large population of a species of *Hydra* developed in the larger streams at the same time as the second generation black-fly larvae. Some of these animals were inadvertently introduced into black-fly rearing bowls in the laboratory, where they rapidly eliminated the larvae. Many specimens, when collected from the streams, were in the process of digesting black-fly larvae; one was taken with an offspring still attached near its base, and parent and offspring each contained a black-fly larva. They were capable of ingesting larvae several times their own initial size; the empty skins were voided from half an hour to an hour afterward. In this season at least, this species of *Hydra* destroyed a very large portion of second generation black-fly larvae. It has not been possible to get living material of the hydra to a specialist for specific identification.

On June 22, 1951, examination of the stomach contents of male and female whitefish, *Coregonus clupeaformis* (Mitchill), showed caddis and midge larvae and corixid adults in both of these, and many large black-fly larvae in the female fish only.

As the season advanced, both in 1951 and in 1952, the proportion of black-fly larvae either containing mermithid parasites or infected by growths of Microsporidia increased until by the last week of July diseased larvae in some places outnumbered the healthy ones. Both *S. venustum* and *E. furculatum* were affected. On June 22, 1951, a mermithid worm was found in a pupa of *S. venustum* in a laboratory colony. Hitherto it has been generally considered that both of these parasites prevent pupation (14, 15). Grunin (5), however, has recently reported adult males and females of *Prosimulium hirtipes* Fries releasing nematodes into streams over which they flew. Two mermithid worms were dissected from the abdomen of an apparently healthy adult female of *S. venustum* collected on July 23, 1951. Several other flies, collected and dissected at the same time, appeared to have no living tissue at all left within the abdomen; there was no alimentary canal, fat body, reproductive system, or nervous system. Some flies affected in this way fed on dyed sugar solutions, which, when the flies were dissected, were found free in the body cavity. Protozoan infections were also found in pupae of *S. venustum* on June 29 and 30, 1951.

A species of oligochaete worm was frequently found associated with pupae, living with these inside the cocoons. What appeared to be the same species of worm was occasionally found in association with egg masses.

Many of the specimens of *S. venustum* collected while laying eggs on July 16, 1951, had larval hydrachnid mites attached to them, one with as many as six in the cervical region.

Birds evidently take heavy toll of adult black flies. On July 11, 1952, a young but fully fledged white-crowned sparrow, *Zonotrichia leucophrys* (Forster), one of the commonest birds nesting in the area, was caged and exposed to black flies in the field to obtain flies that had fed on the blood of this bird. No black flies fed on the bird, even when they were held against

the breast in a vial; a few crawled over the feathers. The bird, however, ate a great many black flies; during one period of five minutes, 54 flies were eaten, most of them *S. venustum*. Several species of mosquitoes, and even the large tabanid *Tabanus affinis* Kby., were also eaten by this bird. Confirmation that this is a general habit was obtained subsequently by watching these birds in the free state.

## Field Rearing Methods

To obtain data on the durations of larval and pupal stages, and to associate larval exuviae with the more readily identified pupae and adults, two methods of rearing selected larvae to adults in the field were tried.

*Trough Method*

Several wooden troughs, square in cross section and 18 in. long by 6 in. wide, were constructed. Slots were provided at both ends to hold wire screens. These troughs were nailed to the wooden piers of the railway bridge over Warkworth Creek so that they projected about an inch above the surface of the water that flowed through them. The downstream end of the trough had two slots so that, before removing one screen to search for larval skins, a second could be inserted. Forty-mesh brass screening was used at this end, 24-mesh at the upstream end. The inner surfaces of the troughs were marked with squares identified by numbers and letters, so that the positions and movements of larvae could be recorded. This method was discarded after a few days' trial; the upstream screen had to be removed and cleaned four or five times daily to maintain a reasonable flow.

*Cage Method*

A rectangular piece of 40-mesh brass screening was rolled into tubular form and the two edges were soldered together. Screw caps from 2-oz. jars were placed over each open end and held in place by means of a U-shaped piece of 12-gauge iron wire, bent so that the ends of the wire pressed into depressions punched in the centers of the caps. The wire thus formed a convenient handle for the cage. These cages were about 1½ in. in diameter and 2 in. in length.

One black-fly larva was placed in each of the screen cages and the cages were anchored under water by cotton threads. They were free to move and rotate so that they were largely self-cleaning. The cages were cleaned daily, both inside and out, with a small stiff brush. This ensured a good flow of water at all times.

When a cage was removed for observation it was brought to the surface of the water and moved up and down several times at the surface level, before the upper end was removed. This washed any head capsules or larval skins into the lower end, where they could be easily seen against the white liner of the screw cap. Observations were made daily.

A small air space under one of the lids of the cages maintained most of them in a vertical position. Emerging black flies were thus able to dry and expand

normally, and specimens in excellent condition were obtained. Some were in such good condition that they flew out when cages were incautiously opened (Table III). Mortality was low. About 80% of the larvae placed in the cages were reared to the adult stage. The main disadvantage was that larvae less than half-grown would pass through the screen so that information on the early stages was not obtained by this means. Head capsules from several of the later larval molts of *E. furculatum* were found. The last stadium for six females of this species ranged from one to five days; the fifth stadium of two of these lasted three days.

Similar cages were constructed of 28-mesh saran screening. A rectangular piece of screening was cut and rolled to make a tubular container 1 in. long by $\frac{1}{2}$ in. in diameter, one end being closed by flattening. A soldering iron was used to fuse the edges together. The open end was closed with a small cork. The 28-mesh size was not fine enough to catch any but the largest head capsules; plastic cages of a finer mesh might, however, prove superior to those of metal.

In an attempt to rear the younger larvae in this way, cages were also made of 100-mesh silk bolting cloth, sewed in the form of tubes 1 in. long and $\frac{3}{8}$ in. in diameter. These were closed with a cork at each end. Several black-fly eggs were placed in each and the cages were submerged in the stream. All the eggs hatched, but no larvae developed beyond the first instar. Mortality may have been caused by the cloth becoming clogged with algae and organic debris so that flow ceased. Cleaning was difficult and involved considerable risk to the very small larvae. In 1952 similar cages were made of 64-mesh tinned copper wire screening. These were better than the silk cages, but even when a large piece of $\frac{1}{4}$ in. wire mesh was fixed just upstream of the cages to screen out the coarser debris, they gave less satisfactory results than the original 40-mesh cages.

The data obtained from these cages are summarized in Table III. The duration of the pupal stage ranged from four to nine days with an average of 5.6 days; there was little difference between sexes or between species.

TABLE III

SPECIES OF BLACK FLIES REARED IN THE FIELD AND DURATIONS OF THE PUPAL STAGE, CHURCHILL, MAN., 1951 AND 1952

| Species | Number reared | | | | Duration of pupal stage, in days | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Male | | Female | |
| | Male | Female | Sex unknown* | Total | Range | Mean | Range | Mean |
| *P. gibsoni* | 2 | 8 | 0 | 10 | 4–4 | 4 | 5–6 | 5.3 |
| *P. decemarticulatum* | 0 | 1 | 0 | 1 | — | — | 4 | — |
| *E. furculatum* | 5 | 11 | 2 | 18 | 5–7 | 6.2 | 4–7 | 5.2 |
| *S. venustum* | 9 | 41 | 6 | 56 | 4–7 | 5.9 | 4–9 | 5.7 |

* *Adults lost on emergence.*

## Laboratory Rearing Methods

*Immature Stages*

The restriction of black-fly larvae to running water and their abundance in rapids and riffles have generally been attributed to their respiratory requirements. Accordingly, most attempts at rearing them in the laboratory have embodied some simple means of bubbling air through the water. Wu (17), however, suggested that the current itself was essential to the nutrition of the larvae. Therefore, a variety of methods of securing both adequate aeration and circulation were tried.

The equipment available consisted of two small air pumps designed for the aeration of aquaria, each driven by a 110-volt, 0.5-amp. motor; fritted stone air breakers for use with these; and a variety of vessels, tubing, etc., such as are found in any temporary field laboratory.

*Bowl Method*

Water circulation was first induced directly by the air stream from the pumps. Two glass capillary air jets were arranged tangentially near the margin of 14-in. white enamel bowls and close to the surface of the water in these, either above or below. Alternatively two air breakers were used, each at the lower end of inclined 1-in. glass tubes, so that the air rising through these tubes carried a current of water with it. With both arrangements a glass or a tin cylinder 5 in. in diameter was placed in the center of the bowl to prevent the formation of a vortex. The maximum current speed obtained with the air breakers was 0.056 f.p.s., and with capillary tubes about twice this. These speeds were less than those of the slowest streams in which larvae were found. About 90% of very young larvae brought in from the steams and placed in stream water in these bowls died within 12 hr.

A 1½-in., two-bladed propeller driven at 1550 r.p.m. by a flexible rubber coupling from the shaft of the motor driving the air pump was then tried. This gave a current speed of 0.75 f.p.s., only a little lower than the average speed of heavily infested streams in the vicinity, and higher than many of these. This bowl was stocked with many very young larvae from Goose Creek on June 9. Most of the larvae promptly attached themselves to the propeller shaft or to the blades of the propeller. Brief stops to investigate this occurrence showed that most of them remained alive and active for about 12 hr., when many began to fall off and some to die. They were moving through the water at linear speeds up to 10 f.p.s.

This suggested that the attachment of larvae is dependent on a steep velocity gradient. As paddle wheels have little velocity relative to the water and do not set up steep velocity gradients, an arrangement in which these were used to create flow was next tried. A shaft carrying flat corks 3 in. in diameter, each with metal blades inserted around the circumference, was driven by a reduction pulley from the same motor. Each of these paddle wheels drove the water in a bowl with a center piece as already described.

The current speed could be adjusted by altering the number and size of the blades. The speed of rotation was 116 r.p.m., and the range of current speeds used was from 0.25 to 0.62 f.p.s. Another paddle-driven bowl was arranged with a smaller paddle, 2 in. in over-all diameter, submerged almost to its axis and driven at 200 r.p.m., giving a current speed of 1.0 f.p.s. At these speeds of rotation some splashing occurred so that frequent replenishment of the water was necessary; larger paddles and lower speeds would be better. Various arrangements of air breakers and vegetation were used to augment the aeration provided by the paddles. Larvae were rarely found attached to the paddles. In 1952 a separate, variable-speed motor was used to drive the paddle wheels, and 3 in. × 1 in. microscope slides were cut in half to make the blades. In some bowls a grid of glass slides or a piece of plastic screening was arranged to create a high velocity gradient and encourage attachment. Few larvae used these, possibly because of their transparency; a visual stimulus may be required to evoke the actual process of attaching.

A procedure (4) used in rearing radioactive individuals for flight studies at the Entomology Laboratory, Saskatoon, was also tried. A bowl was arranged in Warkworth Creek so that the current of the creek drove a large paddle outside the bowl, this in turn driving a small paddle on the same shaft inside the bowl. This method, however, proved less satisfactory than those used in the laboratory.

During the two seasons 63 cultures were started in rearing bowls. No adults were reared from eggs and no first-instar larvae molted to the second instar; however, many adults were reared from larvae of later instars (Table IV).

The cast head capsules of larvae in rearing bowls floated to the edges of the bowls, whence they could be collected. These collections were made daily from the most successful rearing bowl (Table IV, No. 8) and, on the basis of Przibram's factor applied to the mean measurements of daily collections, the average duration of each of the third, fourth, and fifth stadia was calculated to be rather less than three days.

In rearing bowls in which the current speed was low, pupal cocoons were not always spun with the toes upstream. The critical speed for this to occur was about 0.15 f.p.s. At lower speeds orientation of the pupae was random. This was so in the field also. The process of spinning the cocoon and pupating occupied about $1\frac{1}{4}$ hr.

Several stoppages of the aerating and circulating equipment occurred on account of power failure. When this happened most larvae released, and were again confronted with the problem of attachment when power was restored. These occurrences were always associated with a deterioration of cultures.

*Jar Method*

Concurrently with these tests a series of eight cylindrical glass jars 13 cm. high by 5 cm. wide were set up, each with one or more air breakers. These each held 250 cc. of water, and in them a variety of food materials, attachment

## TABLE IV

RESULTS OF ATTEMPTS TO REAR BLACK-FLY LARVAE IN BOWLS, CHURCHILL, MAN., 1951 AND 1952

| Culture No. | Aeration | Circulation | | Initial population | Source and date | Remarks |
|---|---|---|---|---|---|---|
| | | Method | Rate, f.p.s. | | | |
| 1 | 2 glass capillary jets | Glass capillary jets | 0.11 | Many very young larvae | Warkworth Creek June 9, 1951 | 90% mortality overnight |
| 2 | 2 air breakers in inclined tubes | 2 air breakers in inclined tubes | 0.056 | Many young larvae | Warkworth Creek June 9, 1951 | 90% mortality overnight |
| 3 | 2 glass capillary jets | 1½ in. propeller | 0.75 | Many young larvae | Goose Creek June 9, 1951 | Grasses provided for attachment; many still living on June 18 |
| 4 | 1 air breaker | Paddle with small blades | 0.51 | *Ca.* 100 very young larvae | Warkworth Creek June 18, 1951 | Many larvae alive on June 22 |
| 5 | 1 air breaker | Paddle with large blades | 0.62 | *Ca.* 100 young larvae | Warkworth Creek June 18, 1951 | 25 mgm. dried yeast added daily; many alive on June 22 |
| 6 | 1 air breaker and *Sparganium* sp. | Paddle with small blades | 0.25 | Many larvae, various sizes | Warkworth Creek June 18, 1951 | Rocks provided for attachment; many alive on June 22; 6 pupae |
| 7 (*a*) | 3 air breakers | Paddle wheel | 0.42 | Many larvae, various sizes | Warkworth Creek June 22, 1951 | Water cloudy, few larvae alive on June 26 |
| (*b*) | Nil | " " | " | " " " | " " " | Water cloudy, some pupae but no larvae alive on June 26 |
| (*c*) | *Sparganium* sp. | " " | " | " " " | " " " | Water clear, many larvae of all sizes and some pupae alive on June 26 |
| 8 | 1 air breaker and *Myriophyllum* sp. | Small, high speed paddle wheel | 1.0 | *Ca.* 250 larvae of various sizes | Goose Creek June 28, 1951 | *Ca.* 100 cast head capsules collected daily for some days; most larvae pupated and adults emerged; few larvae remained on July 7 |
| 9 | 1 air breaker and *Myriophyllum* sp. | Small, high speed paddle wheel | 1.0 | Several hundred eggs | Warkworth Creek July 7, 1951 | Hatching started July 8; all larvae died in 1st instar by July 14 |
| 10 (*a*) | 1 air breaker | Paddle wheel with glass blades | 0.48–0.7 | Few eggs | Goose Creek July 9, 1952 | Hatching July 10-13; water clear, all larvae died in 1st instar by July 16 |
| (*b*) | *Myriophyllum* sp. | " " " | " | " " | " " " | Hatching July 10-14, water cloudy, all larvae died in 1st instar by July 20 |
| (*c*) | *Myriophyllum* sp. | " " " | " | " " | " " " | Hatching July 10-13, water clear, all larvae died by July 20 |

facilities, and manipulations were used. Moderate circulation was maintained by the stream of bubbles rising from the air breaker. A total of 76 cultures were started in jars during the two seasons. The method proved rather more successful than the bowl method (Table V).

From time to time the oxygen content of water samples from the rearing bowls and jars, and from the creeks and streams, was determined by Winkler's method. In all samples from rearing vessels the oxygen content was close to atmospheric saturation. Sometimes, notably when vegetation was used without an air breaker, it was a little above saturation. Water from the streams showed an average oxygen content a little less than this, and samples from drainage ditches in which larvae were developing contained less still. Air breakers apparently added little oxygen to the water except when the content was very low. The stone type was unreliable; one of a series would suddenly become excessively porous and take all the air supply, leaving the remainder almost without air. The sintered glass type used in 1952 was much better.

Even when larvae were placed in distilled water in sterile jars, food supply did not appear to be a limiting factor in the growth of surviving larvae. After a few days the stomachs of the larvae were filled with what appeared to be fragments from the decayed and distintegrating larvae that had died. Such colonies developed no less well than those in stream water; a profuse growth of algae developed in the water, presumably from inocula introduced with the larvae, although the latter had been rinsed in distilled water. No greater growth rate was detectable when yeast granules were added to the water. The spacing of most of the hairs on the fan rays is such that the yeast cells readily pass between them; groups of them here and there, however, are set close enough to filter out the yeast cells. Although the cells showed no clumping in suspension, no material other than yeast cells could be found in the guts of larvae from such cultures.

Temperature records were kept of the streams and the rearing containers. The diurnal fluctuations in the laboratory were greater than those of the streams. To decide whether this factor was of importance in larval mortality, one of the rearing jars was jacketed with ice at night and with warm water during the day, giving a temperature fluctuation from 32° F. to 82° F. Mortality was no higher than in a check jar.

Since neither food nor oxygen deficiency, nor temperature conditions, could be shown to be responsible for mortality, the possibility that this mortality was due to the accumulation of some toxic product of metabolism in the water was investigated. On July 5, two rearing jars with 10 half-grown larvae each and two with about 100 each were started (Table V, Nos. 10, *a*, *b*, *c*, and *d*). In one of the jars with 10 larvae and one with 100 larvae, the water was changed for fresh stream water every other day by adding this while the previous water was being siphoned out through a screen. Several times the volume contained in the jar was passed through. By July 11, all of the 100 larvae in unchanged water were dead, whereas the

TABLE V

RESULTS OF ATTEMPTS TO REAR BLACK-FLY LARVAE IN JARS, CHURCHILL, MAN., 1951 AND 1952

| Culture No. | Aeration | Water, feeding, and attachment facilities | Initial population | Source and date | Remarks |
|---|---|---|---|---|---|
| 1 | 1 air breaker and vegetation | Creek, 6 mgm. dried yeast daily, vegetation | Many larvae, various sizes | Goose Creek June 14, 1951 | 1 pupa only alive on June 16 |
| 2 | 1 air breaker | Creek, 6 mgm. dried yeast daily, nil | 20 larvae, various sizes | Warkworth Creek June 17, 1951 | 5 larvae alive on June 23; 2 pupae |
| 3 | 1 air breaker | Creek, nil, stones | 20 larvae, various sizes | Warkworth Creek June 14, 1951 | All dead by June 18 |
| 4 | 1 air breaker | Creek, nil, nil | 10 large larvae | Goose Creek June 14, 1951 | 6 pupae by June 16 |
| 5 | 1 air breaker and *Carex* sp. | Creek, half of water replaced daily, *Carex* sp. | Many larvae, various sizes | Warkworth Creek June 17, 1951 | 1 pupa by June 19, most larvae dead by July 21 |
| 6 | 1 air breaker and grasses | Creek, nil, grasses | Many larvae, various sizes | Warkworth Creek June 19, 1951 | Many healthy, half-grown larvae on June 27 |
| 7 (*a*) | 1 air breaker | Distilled, nil, nil | Many larvae, various sizes | Warkworth Creek June 28, 1951 | Many still alive on July 5, much algal growth Larval stomachs full |
| (*b*) | 1 air breaker | Creek, nil, nil | " " " | " " " | Nearly all dead by July 5 |
| 8 (*a*) | 1 air breaker and much *Myriophyllum* sp. | Creek, nil, *Myriophyllum* sp. | Many larvae, various sizes | Warkworth Creek June 28, 1951 | Many larvae and pupae alive on July 8 |
| (*b*) | 1 air breaker and little *Myriophyllum* sp. | Creek, nil, *Myriophyllum* sp. | Many, various sizes | Warkworth Creek June 28, 1951 | Few alive on July 4; all dead by July 5 |
| 9 (*a*) | 1 air breaker | Creek, nil, willow twigs | Many, various sizes | Warkworth Creek June 28, 1951 | Normal daily temp. changes; many alive on July 2 |
| (*b*) | " " | " " " " | " " " | " " " | Temp. 32° F. at night and 82° F. by day; many alive on July 2 |

TABLE V—(*Concluded*)

RESULTS OF ATTEMPTS TO REAR BLACK-FLY LARVAE IN JARS, CHURCHILL, MAN., 1951 AND 1952—(*Concluded*)

| Culture No. | Aeration | Water, feeding, and attachment facilities | Initial population | Source and date | Remarks |
|---|---|---|---|---|---|
| 10 (*a*) | 1 air breaker | Creek, water changed on alternate days, nil | 10 large larvae | Warkworth Creek July 5, 1951 | 1 larva and 4 pupae alive on July 13 |
| (*b*) | " " | Creek, nil, nil | " " " | " " " | 3 larvae and 2 pupae alive on July 13 |
| (*c*) | " " | Creek, water changed on alternate days, nil | 100 large larvae | " " " | Many larvae alive on July 11 |
| (*d*) | " " | Creek, nil, nil | " " " | " " " | Water cloudy and all dead by July 11 |
| 11 (*a*) | 1 air breaker | Distilled, nil, grasses | Eggs on grasses | Warkworth Creek July 11, 1951 | Hatching started July 18, all dead in 1st instar by July 25 |
| (*b*) | " " | Creek, nil, grasses | " " " | " " " | Hatching started July 18, some 2nd instar and 3 half-grown larvae still healthy Aug. 14 |
| 12 (*a*) | 1 air breaker | Creek, nil, grasses | A few eggs | Goose Creek July 11, 1952 | Hatching started July 12, two larvae molted to 2nd instar but died by July 16 |
| (*b*) | " " | Creek, water changed alternate days, grasses | " " " | " " " | Hatching started July 12, only 1 larva alive July 15, 1st instar |
| (*c*) | " " | Creek, nil, grasses | Many eggs | " " " | Hatching started July 12, all larvae died in 1st instar by July 17 |
| (*d*) | " " | Creek, water changed on alternate days, grasses | " " | " " " | Hatching started July 12, web broken by water change on July 13, all dead by July 15 |

majority of those in changed water were alive and appeared healthy. In each of the two jars containing 10 larvae the cultures were in similar condition; in both of them most of the larvae eventually pupated. A repetition of this procedure in 1952 was less conclusive; it is difficult to change the water without breaking larval webs. The frequent demand for reattachment may partially offset the advantage of changing water.

Nearly all of the eggs in masses taken from streams hatched in rearing vessels in the laboratory. The young larvae spun long festoons of webs on which they attached themselves. Although they appeared normal, for some unknown reason few of the larvae molted to the second instar. Field observations suggested that there may be heavy mortality in nature at this stage of development. Cultures containing living vegetation nearly always did better than similar cultures without this.

*Adults*

Although some work was done with adults reared from larvae in the laboratory, most adults used were obtained from pupae collected from streams. Masses of these were brought in on rocks and kept moist in screen cages to allow the adults to emerge.

Only two pairs of *S. venustum* were seen to mate in cages. This was at 0930 hr. on July 7; the males and females had all emerged from pupae collected the previous day. Connection was brief and it is not known whether the matings were effective. The spermathecae from many laboratory-reared females were examined; all were empty. Attempts were made to induce mating by exposing the insects to carbon dioxide as described by Dalmat (1) for various Guatemalan species. A wide range of concentrations and exposure periods was tried without success.

Both male and female flies fed on 25% sucrose solution. When this was colored with various stains and offered on cotton wicks in glass vials at the bottoms of the cages, only a few flies fed heavily, most of them on the red- and orange-colored sirups. When large cotton pads soaked in sirup were placed on top of the cages, where flies spent much of their resting time, feeding was much more general. Finally, when flies were enclosed in Petri dishes with a layer of sirup-soaked cotton, nearly all of them engorged fully. Nectar-bearing flowers placed in the cages were promptly investigated by a small number of flies.

All attempts to induce laboratory-emerged flies to feed on blood were unsuccessful. At various periods after emergence, under various weather conditions, inside and outside of the laboratory, flies were caged on various areas of the human body, and also on dogs, but no blood meals were taken. Furthermore, all flies captured while feeding on the blood of man or of dogs in the field died within 48 hr. of capture.

Attempts to obtain oviposition in the laboratory were unsuccessful. Flies captured, either when about to lay eggs or when actually doing so in the field, laid no eggs when caged over running water containing suitable vegetation

in the laboratory. Specimens of *E. furculatum* taken from laboratory cages where they had no food other than sucrose and water were found on dissection to have up to several hundred well developed eggs in the ovaries.

## Discussion

The proximity of young first-generation larvae to the stream bottom, and their initial appearance in small numbers, suggest that the overwintering eggs of *S. venustum*, like those of *S. arcticum*, accumulate in the beds of the streams, and that they hatch when brought to the surface or into positions of adequate temperature by the shifting of the bed during spring floods. This is supported by the fact that many eggs are laid on deciduous leaves or ephemeral algae, and by the appearance of a second brood with the second spate in 1952.

Observations lend no support to the assumptions that have long been widely made that black-fly larvae attach in rapids and riffles because of nutritional or respiratory requirements. As Nicholson and Mickel (10) have pointed out, the larvae are incapable of swimming. They are, indeed, so completely helpless when freely suspended in water that their arrival at attachment sites can only be brought about by some mechanical action. Everything points to a steep velocity gradient as providing this action; it may be that the effect of this is partially dependent on the unusual and characteristic shape of simuliid larvae. Probably both tactile and visual stimuli are required to initiate the actual attachment process. Once the larvae have attached, a steep velocity gradient is, of course, well suited to their respiratory and nutritional requirements. The pattern of larval attachment already referred to is compatible with a mechanistic explanation. In Fig. 1, *CD* and *DE* represent two portions of an attachment surface inclined at different angles to the current. A uniform distribution of larvae in the water being assumed, the equal elements of the flowing water *A* and *B* would carry equal numbers of larvae per unit of length. These larvae, acted on by the same forces, would be deposited in the densities shown. Spacing is often

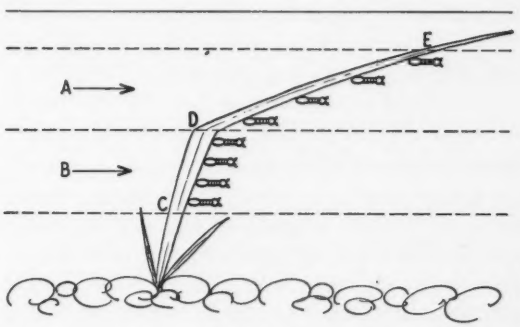


FIG. 1. Pattern of attachment of black-fly larvae, showing dependence on angle of inclination to the current (see above).

strikingly uniform; this doubtless results from voluntary adjustments of relative position after attachment. It minimizes competition for the available food.

The difficulties in the laboratory rearing of *S. venustum* are the molting of first-instar larvae, and mating, blood-feeding, and oviposition. The remainder of the life cycle can be completed with low mortality and little special equipment.

Poisoning by some metabolic waste product may well be a factor in the laboratory mortality of first-instar larvae of the second generation. The same factor would operate in nature, where a similar mortality is suspected, the flow in the streams being at its lowest at this time, when there may still be a large population of mature first-generation larvae. The first generation, especially in the upper reaches of streams, with a large volume of fresh water flowing past them, would not have to face this difficulty. The beneficial effect of plants in laboratory cultures may be due to their utilization of these toxic products in anabolic processes, as well as to improved aeration. As expected from their high surface/volume ratio, very young larvae have no difficulty in obtaining sufficient oxygen. In a mixed population left in still water in the laboratory, the larger larvae become inactive and die long before the small larvae are affected. When transferred to well aerated water, large larvae that have reached the inactive stage soon resume their activities. In general, the greater the volume of water per larva the better it is, from the point of view of respiration, nutrition, and autointoxication. On the other hand, the association of considerable numbers of larvae offers advantages in local movement and in attachment, through the communal web that is spun. The difficulty in laboratory rearing appears to lie in these two antagonistic requirements.

Regarding the difficulties in the adult stage, it seems unlikely that mating swarms of *S. venustum* could have been overlooked; probably this species mates without swarming, shortly after emergence, remaining connected only very briefly, although Nicholson and Mickel (10) have observed swarming "probably of *S. venustum*". No clue can at present be offered to the specific conditions required for the completion of this process or to those required for blood-feeding and oviposition. It is not unlikely that the three processes are interconnected and that, if mating can be induced, the remaining processes will follow.

The energy relationships of black flies seem to be a major factor in their success in arctic and sub-arctic regions. In the larval stage, energy for the bulk of the locomotory activity is provided by the flow of the stream, which also provides food, so that the total energy consumed in the larval stage must be exceptionally small. Willows, with species flowering in sequence and growing close to the larval habitat, provide in their nectar a convenient source of high energy food for adult activities. Females of many species apparently lay eggs without a blood meal, commonly upstream of the emergence site, i.e.,

in a position of higher potential energy. Against this background it seems possible that the mortality in laboratory rearing resulting from power failures is due to an excessive demand for energy for frequent attachments in the larval stage.

## Acknowledgments

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# ON THE SPECIFICITY OF *LEUCOCYTOZOON*[1]

By A. M. Fallis, J. C. Pearson, and G. F. Bennett[2]

## Abstract

*Leucocytozoon simondi* Mathis and Leger was transmitted artificially to a gosling. Other goslings became infected naturally when exposed beside infected ducks. Negative results were obtained from attempts to transfer *L. simondi* artificially to ruffed grouse, chickens, turkeys, and pheasants and no natural infections were detected in these birds that were exposed for weeks beside infected ducks. *L. sakharoffi* Sambon could not be transmitted artificially to ducks, chickens, or a pigeon.

During the last few years blood smears prepared from several hundred birds of different kinds have been examined. Species of leucocytozoon occurred more often than any other parasite. The highest incidence was recorded in the ruffed grouse, and was somewhat higher than the figures previously reported for Ontario (1). Experimental studies on *Leucocytozoon simondi* were made (2) which indicated that ducks rapidly become infected. These experimental birds were exposed in June in Algonquin Park, Ontario, in an area where wild ducks, although scarce, were present, but where there was a greater population of ruffed grouse with a high incidence of leucocytozoon. These observations raised the question of the specificity of the parasites that were observed in different birds. Observations and results of experiments that were designed to test this specificity are reported below.

## Historical

The apparent specificity of members of the genus *Leucocytozoon* has been noted by previous investigators. Mathis and Leger (7) referred to the specificity of the species in domestic fowl in Tonkin. They found a high proportion of chickens infected with *L. sabrazesi* Mathis and Leger and *L. caulleryi* Mathis and Leger but no infection was found in 196 ducks, 72 geese, 23 turkeys, 35 partridge, and 253 pigeons that were kept in the same yard. Stephan (9) failed to find infection in turkeys that were in proximity to infected geese. He transferred blood from an infected goose to a turkey but no parasites were observed in the blood of the turkey when it was examined 24 hr. later. Likewise he failed to transmit infection when blood from an infected goose was injected into a duck, pigeon, and sparrow. Parasites in the blood of geese survived for at least three days when injected intravenously into other geese. Knuth and Magdeburg (5) found infected geese and

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turkeys on the same estate but noted a difference in the appearance of the parasites. Parasites were not observed in two ducks that shared the same yard and pasture as the infected geese. Skidmore (8) found *L. smithi* in each of 40 turkeys that he examined on a farm where a number had died presumably from this infection. No specimens of leucocytozoon were observed in the blood of chickens, ducks, and geese that were raised on the same farm. Leger and Leger (6) discussed the morphological characteristics of *Leucocytozoon* spp. that have been reported in different families of birds. They indicated that the appearance of the parasite may depend on the kind of bird infected. Herman (3) thought his data from a study of the protozoa in birds from Cape Cod showed a relationship between the form of the parasite and the kind of bird that was infected.

## Materials and Methods

Ducks, goslings, grouse, crows, chickens, turkeys, and pheasants were exposed for various periods in the woods at the Wildlife Research Station, Department of Lands and Forests, Algonquin Park, Ontario. Blood smears were prepared from them at intervals, stained with Giemsa, and examined for the presence of parasites. Tissues from birds that showed gametocytes in the peripheral blood were emulsified in saline and injected intraperitoneally into other birds. Likewise specimens of Simuliidae that are known vectors were fed on ducks, crows, and grouse that had gametocytes in the peripheral blood. These flies were macerated in Tyrode's solution at intervals following their blood meal and the resulting suspensions were injected into various birds. The birds receiving these inoculations were tested for infection by examining blood smears that were taken from them daily for one week beginning the seventh day following exposure to infection. Blood containing gametocytes of *L. simondi* was transfused into other birds. Blood smears were made from the latter at intervals following these transfusions to determine whether the parasites had survived.

## Results

*Leucocytozoon sakharoffi* Sambon, *L. simondi* Mathis and Leger, and *L. bonasae* Clarke were known to be present in crows (*Corvus brachyrhynchos* L.), ducks (*Anas rubripes* L., *Mergus merganser* L.), and grouse (*Bonasa umbellus* L., *Canachites canadensis* L.) in this area. In 1948 we exposed 20 pheasants (*Phasianus colchicus* L.), 10 turkeys (*Meleagris gallopava* L.), 9 chickens (*Gallus domesticus* L.), 2 ruffed grouse (*Bonasa umbellus* L.) as well as 41 ducks (*Anser domesticus* L.) in the same area in Algonquin Park, Ontario. All of the ducks became infected with leucocytozoon but parasites were not observed in any of the other birds. New infections developed continually in the ducks during a two month period as shown by the infection that developed in parasite-free ducks that were introduced into the area at regular intervals during this time. In the following year six young turkeys were kept

close to 14 ducks that were exposed to infection for two weeks during late June and early July. Infection developed in all ducks but in none of the turkeys. In 1950 five chickens remained free of infection when exposed for two months beside 50 ducks all of which became infected. A similar observation was made in 1951 when two chickens were kept during June and part of July beside 40 ducks all of which became infected. Similar observations have been made on six ruffed grouse that were kept in 1950 and 1951 beside ducks in which new infections were developing throughout the summer. Parasites were not observed in any of the grouse until the latter part of the summer. Moreover these parasites appeared like *L. bonasae* rather than *L. simondi*.

Two goslings were exposed beside infected ducks and nine days later parasites were observed in the blood of the goslings. These parasites resembled *L. simondi*. These infections had arisen presumably from infected ducks in the vicinity as there were no wild or domestic geese within many miles of the area. This result, together with the results of the cross infection experiment reported below, suggests that *L. anseris* Knuth and Magdeburg is a synonym of *L. simondi*. Herman (4) intimates, in a personal communication, that he proposes to synonymize these species.

The absence of infection in birds other than ducks even though they were exposed in the same area could be related to selective feeding of the black flies. Observations indicated, however, that black flies fed on all birds although perhaps to a greater extent on ducks. In view of the number of birds exposed it appears most improbable that lack of infection in them was a result of black flies feeding on ducks only.

Tissues from a duck containing an infective stage of *L. simondi* were injected intraperitoneally into a gosling and a duck. Low grade infections appeared 10 days later in the blood of each of these birds. The parasitized host cells in both birds were round rather than elongate, as occurs during the first days that infection with *L. simondi* can be detected in the blood of ducks with heavy infections.

The specificity of *L. simondi* was tested further in other experiments involving cross infections. Tissues from ducks that contained an infective stage (presumably merozoites) were injected intraperitoneally into six turkeys, six pheasants, three chickens, and two ducks. Infections were produced in the ducks as a result of these inoculations but not in the other birds.

Similar experiments were carried out in which tissues from ducks infected with *L. simondi* were transferred to ruffed grouse, a chicken, and ducks. Infection developed in the ducks but in neither of two ruffed grouse nor in a chicken.

Tissues from a crow that was infected with *L. sakharoffi* were transferred in a similar way to a grouse, pigeon, and duck in one experiment and to a grouse, pigeon, and chicken in a second experiment. No infection was

observed in any of these birds as a result of these transfers. These two experiments are, by themselves, of limited value for part of the tissue material from the infected crow was not injected at the same time into a control bird such as a crow.

It has been shown (2) that artificial transmission of *L. simondi* is possible sometimes by transferring blood at certain times from an infected duck to a healthy one. Evidence was produced also to show that gametocytes of *L. simondi* survived for several days when transferred to another duck. It was considered advisable therefore to determine whether *L. simondi* could be transferred to a ruffed grouse by an intravenous injection of blood from an infected duck that was harboring gametocytes. One cubic centimeter of blood containing gametocytes of *L. simondi* was injected intravenously into a ruffed grouse. No parasites were observed in a blood smear that was made from this grouse two hours after it had received the gametocytes.

In a second experiment 5 cc. of blood, containing many young parasites as well as mature gametocytes of *L. simondi* from a duck, were injected intravenously into a ruffed grouse that had low grade infection with *L. bonasae*. A similar quantity of blood from the same infected duck was injected intravenously into a parasite-free duck. A blood smear prepared from the grouse 15 min. after the infection showed gametoctyes of *L. bonasae* and many of *L. simondi*. One hour later fewer gametocytes of *L. simondi* could be detected in the grouse and none were observed 16 hr. later. Gametocytes were common in the control duck 16 hr. after the injection and they were present also 16 days later. The gametocytes seen in the control duck 16 days after the injection may have arisen from a new infection that had been produced as a result of the inoculation. A similar infection did not appear in the grouse that had received the injection of blood.

## Summary

The observations and results of experiments indicate that ducks and geese are suitable hosts for *L. simondi* and raise the question of the validity of the species *L. anseris*. Failure to infect ruffed grouse, chickens, turkeys, and pheasants with *L. simondi* indicates that this species is distinct from the other species that have been described from these birds. There are indications also of the specificity of *L. sakharoffi*.

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# *ORNITHOFILARIA FALLISENSIS* N. SP. (NEMATODA: FILARIOIDEA) FROM THE DOMESTIC DUCK WITH DESCRIPTIONS OF MICROFILARIAE IN WATERFOWL[1]

By Roy C. Anderson

## Abstract

*Ornithofilaria fallisensis* n. sp. is described from the domestic duck and compared with closely related species. Four types of microfilariae are described from waterfowl besides that of *O. fallisensis* n. sp.

## Introduction

Several white Pekin ducks, used for studies of *Leucocytozoon* in Algonquin Park, Ontario, became infected with a species of filariid during the spring and early summer of 1953. Two of these birds were killed and subsequent examination revealed the presence of numerous small nematodes beneath the transparent fascia lining the skin. This nematode constitutes a new species and it is described herein and named in honor of Dr. A. M. Fallis, Director of Parasitology, Ontario Research Foundation.

Microfilariae have been found on smears of blood from several species of wild waterfowl from Ontario and elsewhere. Several of these smears contain microfilariae similar to those of *O. fallisensis* n. sp. Others contain microfilariae obviously different. Descriptions and comparisons of these microfilariae follow. In addition, Dr. Norman D. Levine, Professor Veterinary Parasitology, University of Illinois, who with Mr. H. C. Hanson, has recently described two types of microfilariae from the Canada goose (*Branta canadensis*), has kindly loaned me their specimens and these microfilariae are compared with those found in our collection of blood smears from waterfowl.

One species of filariid, *Sarconema eurycerca* Wehr, 1939 (14) from the heart muscle of the whistling swan (*Cygnus columbianus*), has been described from the Anseriformes in North America although the following survey of microfilariae from waterfowl shows that several species are involved.

## Materials and Methods

The adult worms were fixed in a hot mixture of 70% alcohol and glycerine. The alcohol was allowed to evaporate slowly until the specimens remained in pure glycerine, following which they were examined. Most of the microfilariae studied herein were examined on thin blood films that were quickly air-dried, fixed in absolute alcohol, and stained with Giemsa. The microfilaria of *O. fallisensis* n. sp. did not stain satisfactorily with Giemsa, however, and it was necessary to determine the relative positions of the fixed points (pores, special cells, etc.) from specimens stained with methyl green pyronin. The length of microfilariae stained with methyl green pyronin is not significantly

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different from those stained with Giemsa. Therefore the data from the microfilariae of *O. fallisensis* are readily comparable to those from other types of microfilariae stained with Giemsa. The "Mundgebilde" and the "Schwanzgebilde" of Fülleborn (6) were demonstrated in microfilariae stained with azur II-eosin. Table I lists the blood smears from wild waterfowl which were used in this study.

TABLE I

BLOOD SMEARS FROM WATERFOWL CONTAINING MICROFILARIAE IN THE COLLECTION OF THE ONTARIO RESEARCH FOUNDATION

| No. | Common name | Scientific name | Locality | Date |
|---|---|---|---|---|
| 1 | Wood duck | *Aix sponsa* | Manitoulin Is., Ont. | Sept. 14, 1950 |
| 2 | Black duck | *Anas rubripes* | Algonquin Pk., Ont. | July 16, 1952 |
| 3 | Black duck | *Anas rubripes* | Manitoulin Is., Ont. | Sept. 14, 1950 |
| 4 | Lesser scaup duck | *Aythya affinis* | Barrie Is., Ont. | Nov. 11, 1950 |
| 5 | Black duck | *Anas rubripes* | Algonquin Pk., Ont. | June 24, 1938 |
| 6 | Lesser scaup duck | *Aythya affinis* | Wolsey Lake, Ont. | Nov. 12, 1950 |
| 7 | European widgeon | *Mareca penelope* | Scotland | Sept. 20, ? |
| 8 | Lesser scaup duck | *Aythya affinis* | Columbia, S.A. | Mar. 20, 1950 |
| 9 | Pintail | *Anas acuta* | Holland Marsh, Ont. | Oct. 21, 1950 |
| 10 | Mallard duck | *Anas platyrhynchos* | Manitoulin Is., Ont. | Sept. 21, 1950 |
| 11 | Green-winged teal | *Anas carolinense* | Holland Marsh, Ont. | Oct. 6, 1950 |
| 12 | Green-winged teal | *Anas carolinense* | Holland Marsh, Ont. | Oct. 6, 1950 |
| 13 | European teal | *Anas crecca* | Scotland | Sept. 20, ? |
| 14 | Black duck | *Anas rubripes* | Sackville, N.B. | ? |
| 15 | Mallard | *Anas platyrhynchos* | Holland Marsh, Ont. | Oct. 6, 1951 |
| 16 | Lesser scaup duck | *Aythya affinis* | Wolsey Lake, Ont. | Nov. 12, 1950 |

## *Ornithofilaria fallisensis* n. sp.

*Description*

Dipetalonematidae Wehr, 1935; Splendidofilariinae Chabaud and Choquet, 1953; *Ornithofilaria* Gönnert, 1937. The worms, which are exceedingly delicate, are long, slender, and attenuated. The anterior end is round and simple (Figs. 1, 4). The oral opening is a fine dorsoventral slit surrounded by a delicate cuticular border; it opens almost directly into the esophagus. The oral papillae are small and difficult to discern particularily in lateral view.

They consist of one pair of submedian papillae and another pair which are more lateral in position.* The lateral amphids are posterior to the oral papillae. The long, slender esophagus is not divided externally into two portions. The anus is well developed in both sexes (Figs. 5, 6). The cuticle, which is devoid of cuticular bosses, alae, or ridges, is smooth except for a distinct transverse striation which is visible under high magnification.

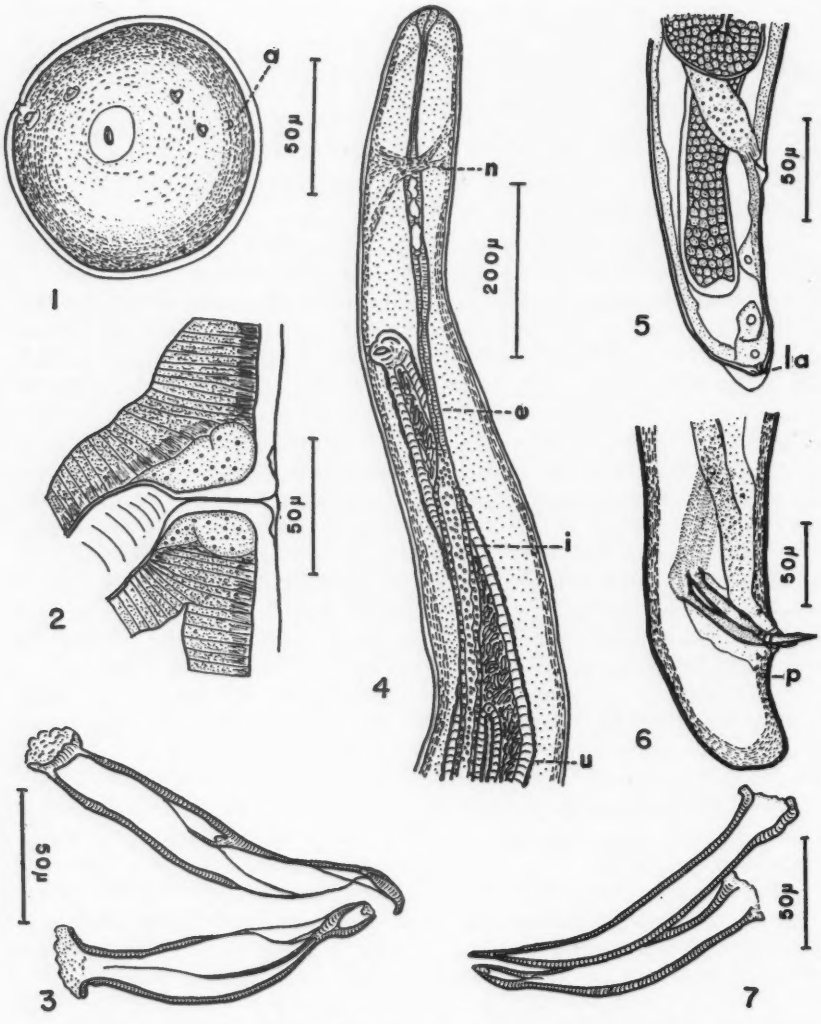
*Male (Seven specimens)*

The males are 9–15 (12) mm. long. The anterior end is 0.037–0.045 (0.039) mm. wide at the point behind the oral opening where the slender part of the esophagus joins the dilated anterior part. The body is 0.053–0.061 (0.057) mm. wide at the nerve ring and 0.053–0.071 (0.060) mm. wide at the anus. The maximum width, 0.092–0.14 (0.11) mm. is attained near the middle of the body. The nerve ring is 0.092–0.14 (0.12) mm. from the anterior end. The esophagus, 0.36–0.60 (0.43) mm. long, is slender, variable in length, and frequently convoluted in the fixed specimens. A slight dilation of the esophagus occurs immediately behind the oral opening. The spicules are subequal and morphologically dissimilar (Figs. 3, 7). The lengths of the right and left spicules of the seven specimens are respectively as follows: 0.072, 0.086; 0.066, 0.091; 0.061, 0.079; 0.065, 0.092; 0.064, 0.072; 0.057, 0.082; and 0.053, 0.076 (Av. = 0.062, 0.082). Both spicules are stout and curved ventrally. The left spicule ends in a blunt point and, in side view, has the appearance of a thick, curved surgical needle. The distal end of the right spicule is broad and spatulate. The spatulate part of this spicule is attached to the main portion of the spicule by a narrow neck. The anus, 0.058–0.079 (0.065) mm. from the tip of the tail, opens on a prominent protuberance and is bordered by a broad transparent cuticular band (Fig. 6). Anal papillae were not found on all specimens. At least three pairs of small postanal and two pairs of preanal papillae are present on those specimens on which papillae were observed. The anal papillae are variable in shape. Usually they are cone-shaped but on one specimen, a single pedunculate papilla was seen along with the usual cone-shaped types (Fig. 6). What appears to be a large, flat, sessile papilla is present immediately in front of the anus. This structure could not be seen in lateral view. The tip of the tail is rounded and provided with a pair of tiny ventrolateral phasmids.

*Female (10 specimens)*

The females are 24–40 (32) mm. long. The head is 0.042–0.050 (0.048) mm. wide. The body is 0.079–0.090 (0.084) mm. wide at the level of the vulva and 0.045–0.068 (0.053) mm. wide at the anus. The maximum width of the body, 0.13–0.23 (0.18) mm., is reached near the middle of the worm. The nerve ring is 0.079–0.13 (0.11) mm. from the anterior end. The esophagus, like that of the male, is slender and extremely variable in length

** The cephalic pattern as determined for this species is at variance with that usually found in the Filarioidea (see Chitwood and Chitwood (4)).*




FIGS. 1–7. *Ornithofilaria fallisensis* n. sp. FIG. 1. Anterior end female, *en face* view. FIG. 2. Vulva and anterior end vagina, lateral view. FIG. 3. Spicules of male, ventral view, slightly distorted by pressure of cover slip. FIG. 4. Anterior end female, ventral view. FIG. 5. Caudal end female, lateral view. FIG. 6. Caudal end male, lateral view. FIG. 7. Spicules of male, lateral view.

Abbreviations in Figs. 1–7.

*a*, amphid
*e*, esophagus
*i*, intestine
*lp*, lateral appendage
*n*, nerve ring
*p*, pedunculate papilla
*u*, uterus.

measuring 0.24–0.50 (0.38) mm. long (Fig. 4). The vulva is 0.20–0.38 (0.26) mm. from the anterior end. The muscular vagina is 0.34–1.0 (0.65) mm. long. The vagina divides into two large, opisthodelphic uteri which are packed with embryos in various stages of development. The uteri in one female measured about 14 mm. long. Fully developed embryos are found coiled within delicate oval-shaped envelopes in the posterior regions of the uteri. The greater and lesser diameter of 15 of these eggs are respectively as follows, 19–25 (23) $\mu$ and 17–19 (18) $\mu$. The intestine of the female is thick and well developed. The rectum likewise is well developed. The prominent anus is 0.050–0.087 (0.073) mm. from the tip of the tail. The tail ends in a blunt point and is provided with a pair of small lateral protuberances (Fig. 5). These protuberances consist of a cuticular cone containing a core of tissue from the region of the body wall; they were not observed on all specimens. The phasmids are ventrolateral in position.

*Microfilaria* (Table II)

Twenty living microfilariae measured 90–121 $\mu$ long, although specimens stained with Giemsa or methyl green pyronin were 67–106 (83) $\mu$ in length. An extremely delicate sheath can be seen on only a few of the specimens stained with Giemsa (Fig. 14) and methyl green pyronin but it is visible on all specimens stained intravitally with azur II-eosin (Figs. 15–18). The sheath

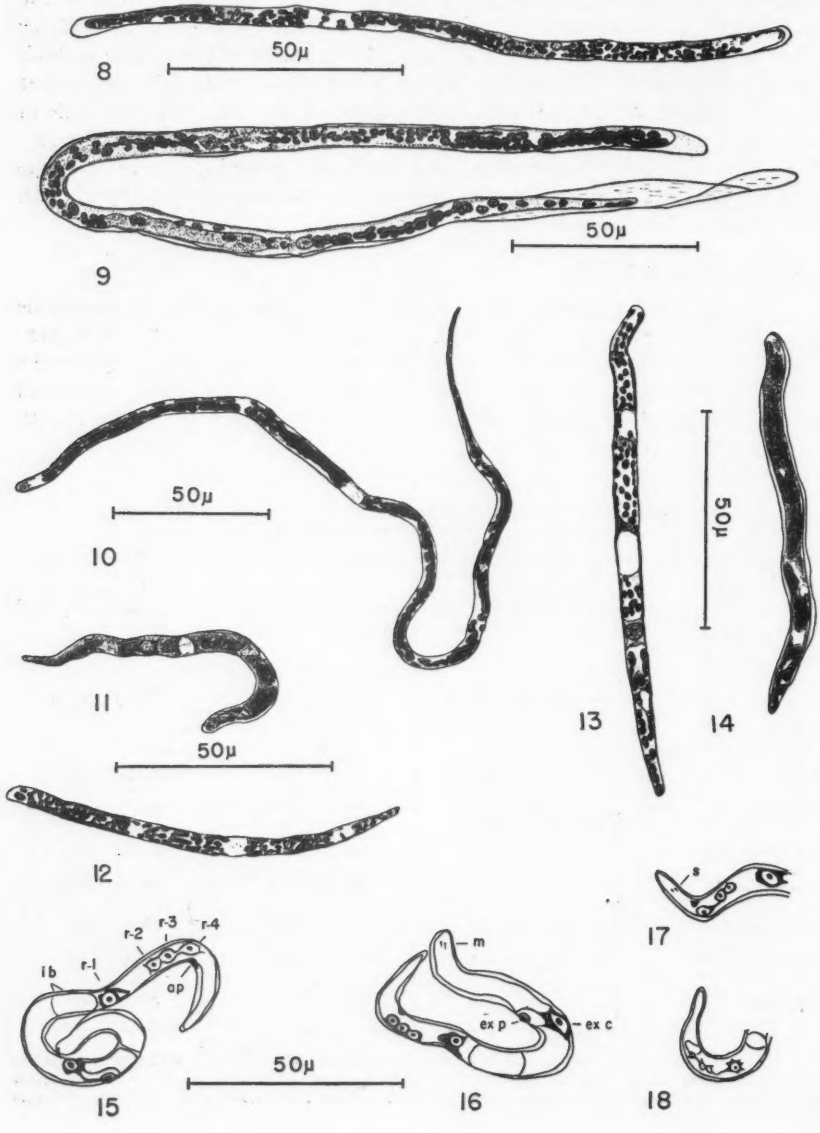
TABLE II

MEASUREMENTS OF THE MICROFILARIAE OF *O. fallisensis* N. SP. AND TWO OTHER TYPES FROM WATERFOWL*

| Host | *O. fallisensis*, domestic duck | Type *B*, black duck (No. 2) | Type *D*, European teal (No. 13) |
|---|---|---|---|
| No. examined | 50 | 10 | 20 |
| Length | 68–106 (83) | 141–155 (152) | 119–138 (128) |
| Cephalic space | — | 0–29 (16)$\mu$ | — |
| Nerve ring | 19–27 (23) | 24–27 (25) | 16–22 (20) |
| Excretory pore | 27–37 (30) | 34–39 (36) | 27–32 (30) |
| Excretory cell | 31–41 (36) | 36–40 (38) | 31–35 (33) |
| Inner body 1 | 47–59 (52) | 60–68 (63) | 49–56 (53) |
| Inner body 2 | 51–65 (60) | 66–72 (69) | 55–62 (60) |
| Rectal cell 1 | 58–76 (68) | 75–82 (78) | 65–73 (68) |
| Anal pore | 77–87 (83) | 88–95 (90) | 80–88 (84) |

** Length is given in microns and other figures are percentages of total length unless indicated otherwise. Inner body 1 and 2 refer respectively to the anterior and the posterior borders of the inner body. The microfilariae of O. fallisensis were stained with methyl green pyronin and other microfilariae with Giemsa.*

is closely applied to the body of the worm. The microfilaria has a serpentine appearance when living but it often appears short and stout on stained smears (Figs. 13–14). The anterior end is rounded. The cephalic space is exceedingly short and usually demarcated from the nuclear column by two lateral, oval nuclei directed along the long axis of the body. The nuclear column



consists of three to four rows of small round nuclei. The nuclei of the tail gradually diminish from about three rows immediately behind the anal pore to a single row of round nuclei extending irregularly along the gently tapering tail. The tail ends bluntly and the final nucleus is located at the tip. The excretory cell and pore were studied on specimens stained with azur II-eosin (Figs. 15–16). The excretory cell is large, in such specimens. Cytoplasmic strands from this cell extend up to and around the pore. There are four rectal cells (Figs. 15–18). The first rectal cell is large, almost the width of the body; the second, third, and fourth rectal cells are much smaller. These latter cells are intimately connected by cytoplasm with one another and they extend to and even slightly past the anal pore. In some specimens the first and second rectal cells are connected by one or two delicate strands of cytoplasm. The inner body ("Innen Körper" of Fülleborn) is large and easily seen. In living specimens it appears as a cylindrical, hyaline body of fluid sharply demarcated from the surrounding cells of the nuclear column. In the fixed and stained material it is represented by a clear space which may or may not be sharply demarcated from the surrounding nuclei. The length of the inner body is highly variable in most fixed and stained specimens although the normal length is well preserved in specimens stained with azur II-eosin. An interesting group of cells can be observed in front of the inner body in specimens stained with methyl green pyronin or azur II-eosin. In the fixed and stained material these cells stain essentially the same as the special cells. These cells, moreover, take up azur II in solution faster than the other cells of the nuclear column except the special cells. The exact number of these cells has not been determined but it is believed to be about four or five. The "Mundgebilde" and the "Schwanzgebilde" of Fülleborn (6) were observed on specimens stained with azur II-eosin (Figs. 16–17).

*Host:* White Pekin duck (*Anser domesticus*).

*Location:* Subcutaneous tissue.

*Locality:* Algonquin Park, Ontario, Canada.

*Specimens:* Zoological Division, United States Bureau of Animal Industry and the Ontario Research Foundation.

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FIGS. 8–18. Microfilariae from Waterfowl. FIG. 8. Type *B* from the black duck (No. 2). FIG. 9. Type *C* from the wood duck (No. 1). FIG. 10. Type *A* from the Canada goose (Illinois). FIG. 11. Short type similar to *O. fallisensis* from the Canada goose (Illinois). FIG. 12. Short type similar to *O. fallisensis* from the black duck (No. 2). FIG. 13. *O. fallisensis* stained with Giemsa and methyl green pyronin; a long extended specimen with sheath not apparent. FIG. 14. *O. fallisensis* stained with Giemsa; a somewhat contracted specimen with a clearly defined sheath. FIGS. 15 and 16. *O. fallisensis* stained with azur II-eosin. FIGS. 17 and 18. *O. fallisensis* stained with azur II-eosin, caudal end.

Abbreviations in Figs. 8–18.

| | |
|---|---|
| *a p*, anal pore | *ib*, inner body |
| *ex c*, excretory cell | *m*, "Mundgebilde" |
| *ex p*, excretory pore | *s*, "Schwanzgebilde" |
| *r* 1–4, rectal cells | |

## Comparisons and Relationships

In 1909 Leiper (8) described *Filaria mavis* from the subcutaneous tissue of *Turdus musicus*. This species was placed, with doubt, in the genus *Aprocta* Linstow, 1883 [Filariidae (Cobbold, 1864) Claus, 1885; Aproctinae n. sf.] by Yorke and Maplestone (18). Gönnert (7) described a filariid removed from *Turdus musicus* from Germany and noted that his material was indistinguishable from Leiper's and also that this species constituted a new genus for which the name *Ornithofilaria* was proposed. Gönnert placed *Ornithofilaria* in the subfamily Aproctinae. Chabaud and Choquet (2) in a recent revision of the superfamily Filarioidea have placed this genus in the family Dipetalonematidae Wehr, 1935 and in their new subfamily Splendidofilariinae. *O. mavis* is apparently the only described member of *Ornithofilaria*.

Morphologically the worms from the domestic duck are similar to *O. mavis* as redescribed by Gönnert (7). The dimensions of the body and the positions and sizes of various structures of the male specimens examined by both Leiper (8) and Gönnert agree with those found in the domestic ducks (Table III). There is, however, little agreement in data derived from female specimens. Table III shows that the females of *O. fallisensis* n. sp. differ from those of *O. mavis* in the following characters: (1) The body is nearly twice as long and twice as slender. (2) The esophagus is only about one half to one third as long. (3) The nerve ring is more anterior. (4) The vulva is more anterior. (5) The vagina is on the average six times as long. These differences are sufficient reason for regarding the worm from the duck as a distinct species.

TABLE III

COMPARISON OF *Ornithofilaria mavis* (LEIPER, 1909) GÖNNERT, 1937, AND *O. fallisensis* N. SP.*

| | *O. mavis* | | *O. fallisensis* n. sp. | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Length | 6.8–11.3 (9.1) | 9.7–21.9 (16.7) | 9–15 (12) | 24–40 (32) |
| Maximum width | 0.21–0.26 (0.22) | 0.26–0.38 (0.32) | 0.092–0.14 (0.11) | 0.13 –0.23 (0.18) |
| Esophagus length | 0.74–1.0 (0.88) | 0.89–1.2 (0.95) | 0.36 –0.60 (0.43) | 0.24 –0.50 (0.38) |
| Nerve ring | 0.13–0.18 (0.15) | 0.13–0.20 (0.17) | 0.092–0.14 (0.12) | 0.079–0.13 (0.11) |
| Left spicule | 76–95 (87) μ | — | 72–92 (82) μ | — |
| Right spicule | 52–83 (73) μ | — | 53–72 (62) μ | — |
| Vulva | — | 0.44–1.0 (0.65) | — | 0.20 –0.38 (0.26) |
| Vagina | — | 0.09–0.19 (0.11) | — | 0.34 –1.0 (0.65) |
| Tail | ——— (0.06) | 0.07–0.13 (0.09) | — | 0.050–0.087 (0.073) |

* *The measurements of O. mavis are from Gönnert (7). All measurements are in millimeters unless indicated otherwise.*

The relative positions of the various fixed points and special cells of the microfilariae of *O. mavis* and *O. fallisensis* are not significantly different. However, the microfilaria of *O. mavis* may be longer. Gönnert (7) gives the measurements of formalin fixed specimens as 121–137 (129) $\mu$ whereas the lengths of living microfilariae (in saline) from *O. fallisensis* n. sp. are 90–121 (105) $\mu$. Gönnert described the microfilariae from the thrush as unsheathed.

According to Gönnert (7) the worms from the thrush were found "... in dichten Knäueln in taschenähnlichen Hohlräumen." He reported that 15 mature worms were found in one subcutaneous pocket. The worms from the domestic duck, on the other hand, were not coiled in knots and there was no evidence of host reaction about them. Moreover, the worms from the ducks were not aggregated but occurred individually. These factors combined with their small size and transparency make this species exceedingly difficult to locate in the duck.

If one follows the recent revision of the Filarioidea by Chabaud and Choquet (2) he might inadvertently place the nematode from the domestic duck in the genus *Paramicipsella* Chow, 1939 (5) or propose a new genus to accommodate this species. Chabaud and Choquet separated the two genera as follows:

"11–(12) Papilles cloacales bien développées
Parasites d'Oiseaux.....*Paramicipsella* Chow, 1939.
12–(11) Papilles cloacales absentes ou plates et peu nombreuses.
13–(14) Vulve au niveau de l'oesophage. Vagin très court, donnant tout de suite deux utérus.
Parasites des Oiseaux.....*Ornithofilaria* Gönnert, 1937."

Gönnert (7) has pointed out, however, that besides finding male specimens of *O. mavis* lacking caudal papillae he found others which had small cone-shaped papillae and one male specimen which had a pedunculate papilla. The same situation has been found in the specimens of *O. fallisensis* n. sp. examined by the writer. Moreover, although Gönnert illustrated a female specimen in which the vagina is very short and divides almost immediately into two uteri, he recorded a range of 0.09–0.19 (0.11) mm. in its length. It is therefore doubtful whether *Ornithofilaria* can be consistently separated from *Paramicipsella* on the basis of the characters laid down by Chabaud and Choquet. Although it is difficult to separate the two genera by these means, the type species of *Paramicipsella*, *P. brevicaudata* Chow, 1939 (5), does, however, have important characters which readily separate it from *Ornithofilaria* spp. They are as follows: (1) The oral papillae consist of four pairs of submedian papillae and a pair of reduced internolateral papillae. (2) There is a rectangular to square cuticular shield at the anterior end. (3) The spicules are equal in size and morphologically similar. (4) The esophagus is short and poorly divided into two portions.

Other species placed in *Paramicipsella*, by Chabaud and Choquet, differ radically from the type species of this genus. For example, at least one species, *P. brevispiculum* (Singh, 1949) (11), has spicules which are unequal in length and morphologically dissimilar. Moreover, a cuticular shield has not

been described apparently in other species in this genus. It would appear therefore that *Paramicipsella* is rather heterogenous at the present time and several of the species require further study before their relationship to *Ornithofilaria* spp. will be clear.

Microfilariae have been reported in the blood of domestic waterfowl in North America at least twice (3, 16). Microfilariae similar to those of *O. fallisensis* n. sp. have been briefly described from several species of wild waterfowl. Most of these microfilariae have been described as unsheathed (9,10,12,15,17). However, the sheath on the microfilaria of *O. fallisensis* n. sp. has been observed only rarely on specimens stained with Giemsa, although it can usually be seen on specimens stained with haematoxylin–eosin. It is possible therefore that the microfilariae of some authors do in fact have sheaths. Furthermore microfilariae which are indistinguishable from those of *O. fallisensis* n. sp. have been found on several of the blood smears from waterfowl in our collection. A list of the hosts together with the number of the slide (see Table I) are as follows: (1) black duck—Nos. 3, 5, 14; (2) lesser scaup duck—Nos. 4, 6, 8, 16; (3) European widgeon—No. 7; (4) pintail—No. 9; (5) mallard—Nos. 11, 15; (6) green-winged teal—Nos. 11, 12. Certain differences in the microfilariae on some slides can be attributed to variations in fixing and staining (Figs. 11, 14).

## Other Microfilariae in Waterfowl

Four types of microfilariae have been found on the slides examined besides those already mentioned which are similar to the microfilaria of *O. fallisensis* n. sp. Since a knowledge of microfilariae can be useful in directing attention to the presence of undescribed filariids in birds, brief descriptions of these microfilariae are given here. For completeness, the writer includes in his classification the long slender form described by Levine and Hanson (9) from the Canada goose.

*Type A* (*Fig. 10*)

*Description*

Unsheathed. The body is long and slender, rounded at the anterior end but tapering to a delicate point at the caudal end. The cephalic space is longer than it is broad. The nuclear column consists of two rows of large, round, or oval nuclei. An inner body is present. The tail is long and tapering, consisting of several rows of nuclei in the region of the anal pore. Only a single row of cigar-shaped nuclei are present in the slender caudal end of the tail. The last nucleus is at the tip of the tail.

*Host:* Canada goose (*Branta canadensis*).

*Locality:* Horseshoe Lake Game Refuge, Illinois, U.S.A.

*Comments:* This form has been described by Levine and Hanson (9) and the reader is referred to their paper. This microfilariae may be the same as that reported from *Mergus serrator* (9,17). Wickware (16) reported, but did not describe, microfilariae in a Canada goose from Ottawa, Canada.

*Type B (Fig. 8, Table II)*

*Description*

With a delicate sheath which is only perceptible at the extremities and at points of constriction of the body. The anterior end is round and has a prominent cephalic space which is usually longer than it is wide. The nuclear column is composed of three or sometimes four rows of round nuclei. The tail is short and barely tapering. The tip of the tail is very blunt and rounded. The tail contains two rows of round nuclei which continue to the tip of the tail. A long inner body is present.

*Host:* Black duck (*Anas rubripes*)—Slide Nos. 2 and 5 (see Table I).

*Locality:* Algonquin Park, Ontario.

*Comments:* Black duck No. 5 also had microfilariae like those of *O. fallisensis* n. sp. Beaudette (1) reported but did not describe a sheathed microfilariae in a "wild duck" from Long Island, U.S.A.

*Type C (Fig. 9, Table IV)*

*Description*

With a coarse sheath extending prominently over the extremities, especially the tail. The body is long, thick, and robust, tapering to a blunt point at the caudal extremity. The anterior end is broad and somewhat flat.

TABLE IV

MEASUREMENTS* OF MICROFILARIAE, TYPE *C* FROM WOOD DUCK (No. 1)*

| | 1 | 2 | 3 | 4 | 5 | 6 | Averages |
|---|---|---|---|---|---|---|---|
| Length plus sheath | 335 | 335 | 350 | 326 | 368 | 327 | 340 |
| Anterior sheath | 6 | 28 | 0 | 9 | 2 | 0 | — |
| Posterior sheath | 26 | 25 | 33 | 34 | 25 | 10 | — |
| Body length | 304 | 282 | 317 | 284 | 340 | 318 | 307 |
| Maximum width | 7 | 8 | 7 | 7 | 7 | 7 | 7 |
| Cephalic space** | 7 | 8 | 7 | 8 | 7 | 9 | 8 |
| Nerve ring** | — | — | 72 (23) | 65 (23) | — | 73 (23) | 70 (23) |
| Excretory pore** | — | — | 106 (34) | 100 (35) | — | 109 (34) | 105 (34) |
| Rectal cell** | — | — | — | 210 (74) | — | 241 (76) | 226 (75) |
| Anal pore** | 259 (85) | — | — | 242 (85) | 287 (84) | 277 (87) | 266 (86) |

** Figures in parentheses are percentages of total body length and all other figures are in microns. The measurements of cephalic space, nerve ring, excretory pore, rectal cell, and anal pore are from the body proper and do not include the sheath.*

*** Distance from anterior end.*

A cephalic space is present. The nuclear column consists of three to four rows of round nuclei. The tail contains a single row of irregularly spaced nuclei. The final nucleus extends nearly to the tip of the tail.

*Host:* Wood duck (*Aix sponsa*)—Slide No. 1.

*Locality:* Manitoulin Island, Ontario.

*Comments:* These microfilariae, although easily distinguished from the preceding, are in a poor state of preservation, hence the data given in Table IV are fragmentary. Nelson and Gashwiler (10) reported a "... large sheathed type (125 microns long by seven microns in diameter)" from a wood duck from Maine. The length given by these writers is much shorter than that found in the specimens studied here and it is probable that two species are involved.

*Type D (Table II)*

*Description*

Like the microfilariae of *O. fallisensis* n. sp. except that it measures 110-138$\mu$ long on blood films stained with Giemsa. A sheath was not observed.

*Host:* European teal (*Anas crecca*)—Slide No. 13.

*Locality:* Scotland.

*Comments:* The length of the above form is greater on stained smears in many cases than that of living material from the domestic duck. These data suggest that a different species of filariid was parasitizing this European teal. Nelson and Gashwiler (10) reported an unsheathed microfilariae, 115–135$\mu$ long, from the wood duck. Wetmore (15) reported an unsheathed microfilaria from the mallard which measured 116–175 (150) $\mu$ long.

KEY TO MICROFILARIAE STUDIED HEREIN

1. Sheath loose, coarse, extending prominently over extremities.....Type *C*
   Sheath absent or if present, delicate and closely applied to body of worm..... 2
2. Body long and slender; tail long, tapering to a delicate point.....Type *A*
   Body shorter, tail short, stubby, and gently tapering..... 3
3. Tail rounded, ending in parallel rows of nuclei.....Type *B*
   Tail bluntly pointed, ending in a single row of scattered nuclei..... 4
4. Length on stained blood films 69–106 $\mu$.....*O. fallisensis* group
   Length on stained (Giemsa) smears 119–138 $\mu$.....Type *D*

## Summary

*Ornithofilaria fallisensis* n. sp. is described from the white Pekin duck. This species differs from *O. mavis* (Leiper, 1909) Gönnert, 1937, in the following features: (1) The female body is much longer and more slender. (2) The esophagus is much shorter. (3) The nerve ring in the female is more anterior. (4) The vulva is more anterior. (5) The vagina is much longer.

The following four types of microfilariae are described from waterfowl besides those of *O. fallisensis* n. sp.

*Type A.*—A long, slender, unsheathed microfilaria with a long tapering tail.

*Type B.*—Microfilaria with a delicate sheath and rounded extremities; tail gently tapering.

*Type C.*—Microfilaria with a large coarse sheath, extending over the extremities; tail long and bluntly pointed.

*Type D.*—Microfilaria like that of *O. fallisensis* n. sp. but somewhat longer.

## Acknowledgments

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